Necrosis Avid Contrast Agents

Functional Similarity Versus Structural Diversity

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Abstract: Two categories of necrosis-avid contrast agents (NACAs), namely porphyrin- and nonporphyrin-based complexes, have thus far been discovered as necrosis-targeting markers for noninvasive magnetic resonance imaging (MRI) identification of acute myocardial infarction, assessment of tissue or organ viability, and therapeutic evaluation after interventional therapies. In addition to necrosis labeling, other less-specific functions, such as first-pass perfusion, blood pool contrast effect, hepatobiliary contrast enhancement (CE), adrenal and spleen CE, and renal functional imaging, also are demonstrated with NACAs. Despite various investigations with a collection of clues in favor of certain hypotheses, the mechanisms of such a unique targetability for NACAs still remain to be elucidated. However, a few things have become clear that porphyrin-like structures are not necessary for necrosis avidity and the albumin binding is not the supposed driving force but only a parallel nonspecific feature shared by both NACAs and non-NACA substances. Although the research and development of NACAs still remain in preclinical stage at a relatively small scale, their significance rests upon striking enhancement effects, which may warrant their eventual versatile clinical applications. The present review article is intended to summarize the cumulated facts about the evolving research on this topic, to demonstrate experimental observations for better understanding of the mechanisms, to trigger broader public interests and more intensive research activities, and to advocate, toward both academics and industries, further promotion of preclinical and clinical development of this unique and promising class of contrast agents.

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Magnetic resonance imaging (MRI) has evolved rapidly into a major player in the armamentarium of clinical imaging diagnoses because of its multiple inherent advantages. Despite this, it is now of no doubt that only when complemented with the use of contrast agents (CAs), MRI can fully play its pivotal role in clinical diagnosis and therapeutic decision-making. The extracellular fluid (ECF) space CAs, such as Gd-DTPA (ie, Magnevist, Berlex Laboratories, Wayne, NJ), have been widely applied for enhancing MRI contrast in both clinical practice and experimental research because of their immediate availability and excellent safety. However, for instance, in the field of cardiac MRI, despite the considerable consensus regarding these ECF CAs as viability markers with "necrosis-specific" property to discriminate between viable and nonviable myocardium at delayed phase contrast enhanced MRI,[1–4] inaccuracy, uncertainty, and dependency of using them on multiple influential factors for imaging interpretation also have been evidenced.[5–9] In particular, they are still incapable of making explicit distinctions between reversible and irreversible injured myocardium, between acute and chronic myocardial infarction (MI), and between ischemic and inflammatory lesions. Such imperfect competence may satisfy some of the present clinical needs but will neither meet the ever-raising healthcare requirements nor match the pace of ever-advancing MRI technologies. Therefore, there has been a continuing strategy for searching more specific CAs that can always offer unambiguous and indisputable imaging diagnosis, of which a particular branch is addressed herewith. Differing from an ordinary research paper, the present article has been structured as a mini-review, in which a few subtitled sections have been organized according to the chronological order of the events with regard to exploration of a unique type of

targeting contrast agents, namely necrosis-avid contrast agents or NACAs.

PREVIOUS EFFORTS IN SEARCHING NECROSIS-SPECIFIC MARKERS

During the past couple of decades, necrosis imaging has been one of the focused interests, particularly in nuclear scintigraphy,[10] which also has affected the research in MRI. Phosphonate-modified Gd-DTPA complexes could produce a persistent and strong contrast enhancement (CE) in diffuse and occlusive MI as the result of their affinity for calcium-rich tissues and subsequent formation of insoluble calcium phosphate precipitates in the damaged myocardium. However, they may cause calcium-homeostasis disorder and consequently impair ventricular contractility.[11] Besides, studies with technetium-99m pyrophosphate, a scintigraphic analog of this type, have shown a lack of specificity between ischemic and necrotic myocardium,[12] leading to a significant overestimation of the infarct size.[13] Antimyosin-antibody labeled magnetopharmaceuticals represent another appealing approach. However, the unaffordable costs, possible immunogenic side-effects, insufficient expression of antigens and limited MRI sensitivity to the currently available relaxation enhancers, and complexity in preparation and handling of the antibody-agents all challenge their ultimate clinical utility.[14]

DISCOVERY OF PORPHYRIN-BASED NACAS

What do x-rays, nylon, and vaccination have in common? They were discovered serendipitously or by accident. The word "serendipity" was first introduced in the middle of 18th century to express the phenomenon of discovery "by accident and sagacity."[15] What likely also belongs to this type is the discovery of another category of necrosis targeting CAs, which represents an ongoing multiepisode story.

Porphyrin derivatives have been investigated for decades in the diagnosis and treatment of malignant tumors.[16–19] The rationales governing porphyrin-mediated cancer photodynamic therapy are based on "tumor-localizing" and photosensitizing properties of the agents. By analogy, the tumor "preferential uptake" of porphyrins also has been exploited for developing paramagnetic metalloporphyrins as "tumor-seeking" MRI CAs.[20–31]

However, the research activities from this laboratory have led to changing metalloporphyrins from being used as tumor seeking CAs into magnetic markers of acute MI.[32] During the early 1990s, in an attempt to screen and confirm a few potentially tumor-specific porphyrin CAs including bis-Gd-DTPA-mesoporphyrin (later renamed as gadophrin-2) and Mn-tetraphenylporphyrin (Mn-TPP) produced and provided by the former Institut für Diagnostikforschung, Berlin, Germany,[28] we conducted a series of experiments on well-established animal models of primary and secondary liver tumors.[33] By using the methodologies dissimilar to those in the previous studies,[16–31] we found that the reported "specific" CE could be attributed only to nonviable (typically necrotic) instead of viable tumor components,[34] an observation just opposite to the assumption raised by an earlier study.[28] To support our findings and to convince people that porphyrins are indeed tumor-nonspecific, more metalloporphyrins were assessed in animals with various induced "benign" necroses and the so called "tumor-localizing" phenomenon could be reproduced without exceptions in these nontumoral lesions.[35] Therefore, certain nonviable tissues (typically necrosis) are thus explicitly identified as the real targets of the studied paramagnetic porphyrin CAs, whereas other intact organs and tissues including viable tumor parts were only enhanced nonspecifically with these agents being treated as certain chemical metabolites.[8,32,34,35] Indeed, necrosis-specific CE and tumoral nonspecific CE could frequently be confused or admixed in animal studies due to most likely the methodological limitations.[34,36,37]

To distinguish from other antibody or receptor mediated tissue specific CAs that feature apparently different mechanisms of targetability, we proposed to nominate these newly discovered porphyrin compounds and later developed nonporphyrin species as necrosis-avid contrast agents, or NACAs, because of their extraordinary avidity to necrotic and/or infarcted tissues.[8,38–43]

GENERAL PERFORMANCE OF PORPHYRIN-BASED NACAS ON CONTRAST-ENHANCED MRI

Although, unfortunately, these porphyrin-based CAs can no longer be considered tumor-specific, their superb necrosis targetability has elicited novel and even more exciting utility for MRI visualization of acute myocardial infarction[8,38–48] and brain infarction.[49] After a few years' experience of peer-suspicion or reluctance likely as one of the common manifestations of the so-called "NIH" (Not Invented Here) syndrome in the academic circle and the industry,[50] eventually the potent effects of gadophrin-2 for labeling necrotic myocardium on MRI have been widely recognized after multi-institutional reproducibility studies.[6,51–57] In addition to intravenous doses of porphyrin–NACAs at 0.05–0.1 mmol/kg for cardiac MRI to visualize acute MI with an extended imaging window during 3–48 hours,[6,40–47,51–57] intracoronary delivery of gadophrin-2 at a tiny dose of 0.005 mmol/kg in combination with the percutaneous transcatheter coronary angioplasty procedure could function as a diagnostic adjuvant for myocardial viability determination and therapeutic assessment for this common cardiac intervention.[8,38,48] Such a smart approach was rated by the French experts as one of the best cardiac imaging techniques in 2002.[58] Nevertheless, improper methodologies often may lead to invalid study conclusions about porphyrin-based NACAs for their applications in experimental MI, causing either the undervalued

capacity for enhancing the occlusive MI[59] or the inaccurate delineation of MI when comparing with ECF CAs,[60] as indicated in the more recent literature.[43,61,62] Other than spontaneous necroses, such as acute MI, porphyrin-based NACAs also could label tissue death after interstitial thermal therapies including radiofrequency ablation (RFA) of solid tumors.[63,64]

So far, triphenyltetrazolium chloride (TTC) staining has been used as the only gold standard for macroscopic identification and quantification of acute MI. However, it is a postmortem technique and hardly applicable in clinic. Studies with both intravenous and intracoronary NACA injections have revealed that actually what is specifically enhanced on cardiac MRI corresponds exactly to what TTC dye does not stain on the excised heart, resulting in the same accuracy for MI delineation.[6,8,39–48,51–57] The measured local concentration of gadolinium is frequently tens of times higher in infarcted over normal myocardium. Experimentally, gadophrin-2-enhanced MRI has been used as a reverse surrogate of TTC histochemical staining or an in vivo viability gold standard for evaluation of medicinal myocardial protection[57] and interventional RFA.[63,64] By chelating a copper ion in the center of the cyclic tetrapyrrole ring, gadophrin-3 has been introduced to improve its structural stability and safety yet still retain its targeting efficacy.[60,62,65] Novel applications of porphyrin-based NACAs in the preclinical experiments on cardiovascular, oncological and even molecular imaging topics are still emerging from different research centers.[57–67] Except for slight discoloration that faded considerably over the course of 24 hours, during animal experiments no detectable side effects were reported with porphyrin agents at a 0.05–0.1 mmol/kg dose range.[6,38–47,49,51–65] Nevertheless, despite optimistic expectations,[66] further commercial development of these colored porphyrin complexes has unfortunately been abandoned by the industry (Weinmann, Schering AG, personal communication), most likely because of the predicted unsatisfactory clinical tolerance and adverse effects upon the unchangeable natures of these dark pigments (Fig. 1).

FURTHER DEVELOPMENT OF NONPORPHYRIN-BASED NACAS

To overcome the discoloration, phototoxicity and other side-effects related to the use of porphyrin derivatives, we have made our continuing efforts to search for more-effective, less-toxic, and less-colored compounds. First, to verify whether the cyclic tetrapyrrole structure characteristic of all porphyrins is essential or not for the observed necrosis targeting, we checked more metalloporphyrins and found that 4 of 9 metalloporphyrins did not prove necrosis avid.[68] Such unequal performances among different porphyrins, also occurring in cancer photodynamic therapy[16,17,19] and tumor imaging,[28] suggest that the tetrapyrrole ring does not appear to be a common structural requirement for the specific targetability. Furthermore, other Gd-chelates conjugated to either open chain tetrapyrroles such as bilirubin and biliverdin or smaller constituents such as mono-, bis-, and tri-pyrrole derivatives, also failed to convincingly reveal a necrosis-specificity.[41] These findings not only disprove an inevitable linkage between porphyrin-related structures and the affinity to necrosis but also implicate the possibility to generate some totally different nonporphyrin molecules that could be more effective and less colored or even colorless and, therefore, deprived of any unwanted effects associated with porphyrins. After a rational roadmap with certain conceptual breakthroughs, we have been able to successfully synthesize a few promising leading compounds such as the light yellowish ECIII-60 (bis-Gd-DTPA-pamoic acid, Fig. 2) and the colorless ECIV-7 (bis-Gd-DTPA-bisindole, Fig. 3),[41,69,70] with both featuring extraordinary necrosis avidity (Fig. 4). The former is derived from pamoic acid, which is a common matrix for pharmaceutical preparations,[71] whereas the later indole derivative partially simulates catabolic metabolites of organisms,[72] and both are presumably more biocompatible than those manufactured materials.[73] Some physicochemical features of porphyrin and nonporphyrin NACAs are compared in table 1.

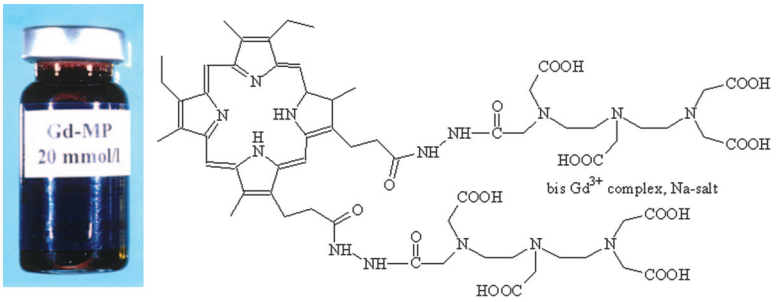

FIGURE 1. A vial containing gadophrin-2 at a concentration of 20 mmol/L shows a nontransparent dark color (left) and its corresponding chemical structure (right).

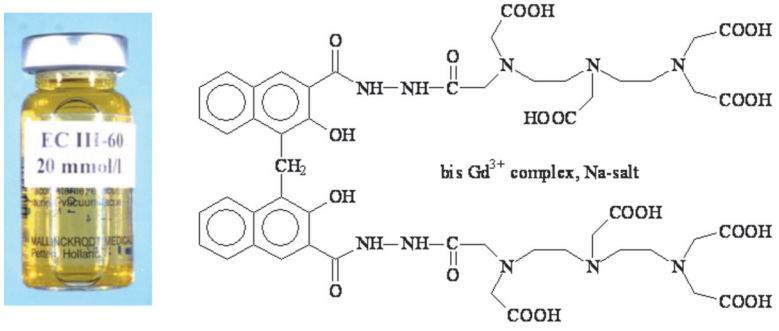

FIGURE 2. A vial containing the nonporphyrin NACA ECIII-60 (bis-Gd-DTPA-pamoic acid derivative) at a concentration of 20 mmol/L shows a transparent light yellow color (left) and its corresponding chemical structure (right).

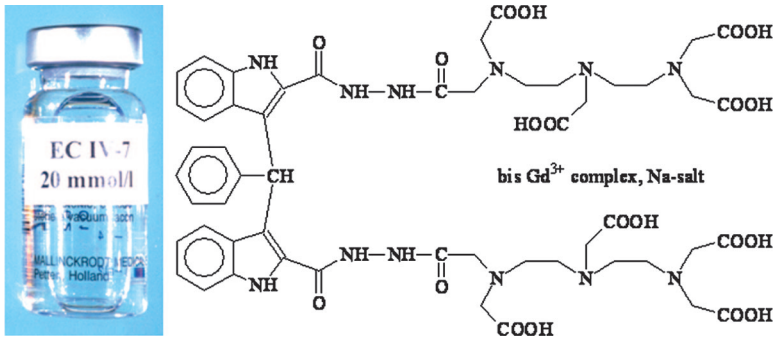

FIGURE 3. A vial containing the nonporphyrin NACA ECIV-7 (bis-Gd-DTPA-bis-indole derivative) at a concentration of 20 mmol/L appears as a transparent colorless solution (left) and its corresponding chemical structure (right).

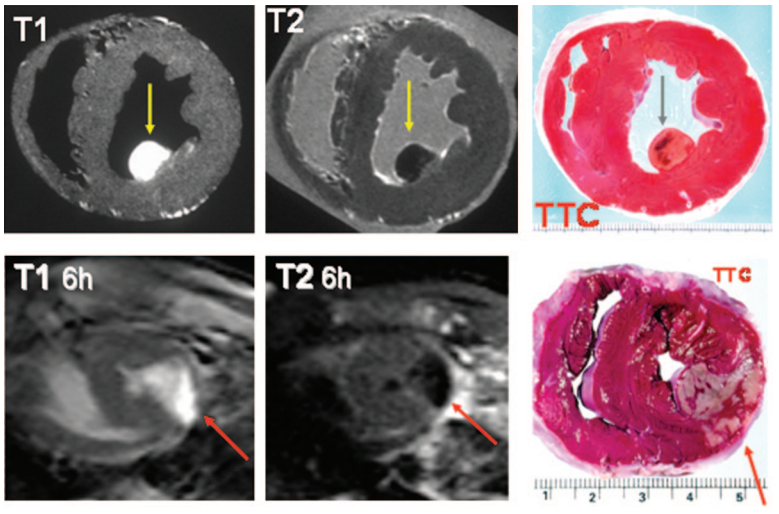

FIGURE 4. Nonporphyrin NACAs at the same intravenous dose of 0.05 mmol/kg induce both T1 and T2 CE with MRI relevant sequences in reperfused MI on postmortem images of a dog overnight after injection of ECIII-60 (top row) and on in vivo images of a pig 6 hours after injection of ECIV-7 (bottom row), suggesting the chemotactic accumulation of NACAs in the necrotic myocardium as proven by the corresponding TTC stained specimens. The imaging was performed at a 1.5-T magnet.

MULTIFUNCTIONAL FEATURES OF NACAS

All studied NACAs, whether porphyrin or nonporphyrin species, allowed differential diagnoses between reversible ischemic injury and irreversible infarct, acute and healing MI, and occlusive and reperfused MI.[6,8,38–49,51–62,66] Even negative findings after CE with NACAs help to reliably exclude the presence of necrosis and reaffirm tissue viability, which would also be of high significance for differential diagnoses.[38,43] Local high concentrations of chemotactically accumulated NACAs enabled both T1 and T2 CE in reperfused MI at relevant MRI sequences, suggesting their extraordinary bifacial capacities (Fig. 4). In a recently proposed "one-stop-shop" comprehensive package of cardiac MR for myocardial viability assessment, the NACA serves as the only key factor that can provide a clear-cut distinction between viable and necrotic myocardium, which is crucial for stratification of patients with acute coronary syndrome and subsequent therapeutic planning in potential clinical applications.[38]

On the other hand, an urgent need for NACAs in therapeutic assessment after RFA of malignant tumors to

TABLE 1. Features of Some Necrosis Avid Contrast Agents for MRI

Name	Generic Name	MW (Dalton)	Color	Plasma Half-life	Excretion	References
Bis-Gd-DTPA-mesoporphyrin	Gadophrin-2, Gd-MP	1697	Dark red	~2.5h*	Urine and bile	6,8,28,32,34,35,38–49,51–59, 61,63,64,66–68,74,88,89
Bis-Gd-DTPA-mesoporphyrin-Cu	Gadophrin-3	1759	Dark red	2.0h	Urine and bile	60,62,65
Mn-tetraphenylporphyrin	Mn-TPP	1111	Green	~2.5h	Urine and bile	28,32,34,35,44,45,47,66,68
Bis-Gd-DTPA-pamoic acid derivative	ECIII-60	1560	Yellow	~2.5h†	Urine and bile	41–43,69,91
Bis-Gd-DTPA-bis-indole derivative	ECIV-7	1582	Colorless	~2.5h†	Urine and bile	41–43,70,74,75,78,79,91

*From the dog.
†From the pig.
MW, molecular weight.

differentiate residual tumor tissue and periablational benign reactive tissues has been recently emphasized.[74,75] Although most unlikely achievable with the use of less tissue specific CAs such as macromolecular blood pool CAs or commercial ECF CAs,[75–77] the use of new NACAs in this regard may fundamentally solve the problems posed by the 2 recent articles.[76,77] Thus, functioning as a virtual biopsy technique, the resultant NACA-enhanced MRI would provide unconditional and unambiguous imaging outcomes for physicians to make early differential diagnosis and therapeutic adjustment,[74,75,78,79] hence higher cure rate for this type of anticancer therapies. Studies with a new nonporphyrin NACA has demonstrated that with the nonspecific liver CE gradually diminishing from a few hours to a few days postcontrast, a specific rim or "O"-type CE around the RFA lesion indicates a complete tumor ablation, whereas an incomplete rim or "C" type enhancement with moderately discernible contrast at the residual viable tumor suggests an incomplete tumor ablation.[74,75,78,79] Therefore, NACAs are advantageous over any other existing contrast agents for this particular application because of their characteristic CE and optimal phase in relation to the cell type (malignant or benign) and tissue viability (living or necrotic).[74,75,78,79]

In addition to the necrosis-targeting property, NACAs also share some exploitable features commonly seen with other existing CAs,[1–5,80,81] for instance, their relatively long plasma half-life as the result of protein binding facilitates their utility as blood pool CAs for MR angiography (Fig. 5), especially of coronary arteries; their amphiphilicity as well as hepatobiliary and renal excretion pathways may render applications for liver and kidney specific CE (Fig. 6). Therefore, with combined specific and nonspecific capacities, NACAs may serve well as versatile or multipurpose contrast-enhancing agents.[42] A similar example can be found with Gd-BOPTA or

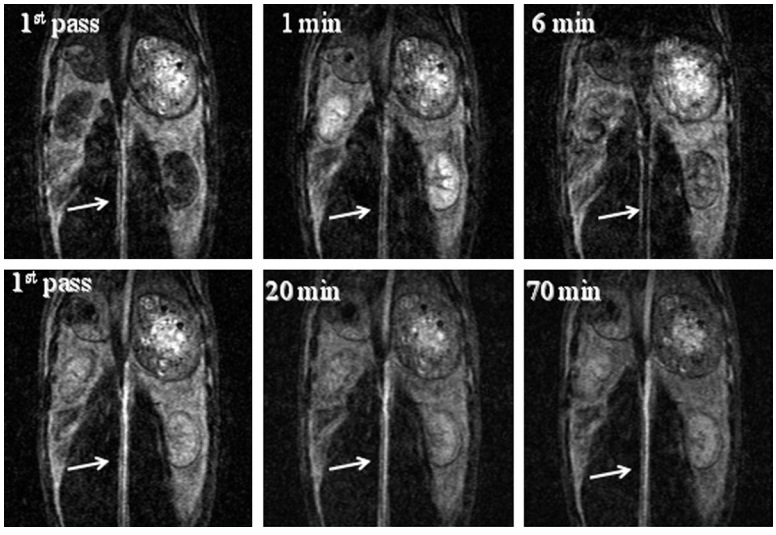

FIGURE 5. MR angiography of rabbit aorta (arrow) at 1.5 T comparing Gd-DTPA at 0.1 mmol/kg (top row) and the nonporphyrin NACA ECIV-7 at 0.05 mmol/kg (bottom row) displays rapid clearance of Gd-DTPA from the circulation and BP effect of ECIV-7 over the course of 70 minutes.

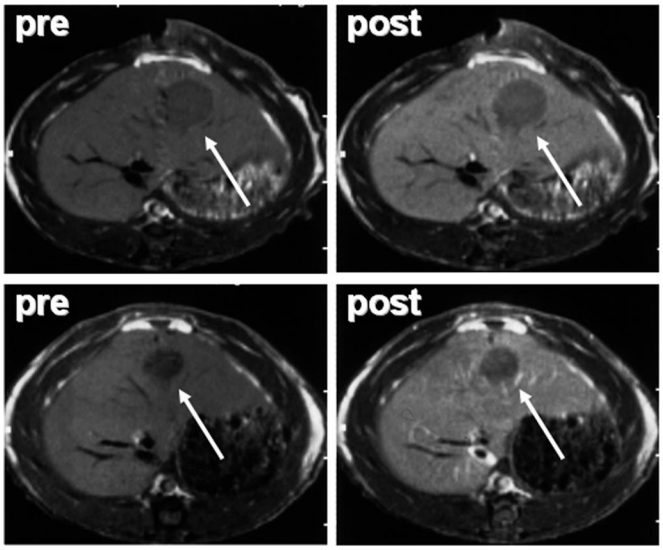

FIGURE 6. Ten minutes after intravenous injection, both the liver specific CA Mn-DPDP (top row) and the nonporphyrin NACA ECIV-7 (bottom row) at the same dose of 0.01 mmol/kg enhance the tumor conspicuity on T1-weighted MR images in the rats with liver implantation of rhabdomyosarcoma, suggesting additional hepatobiliary CE function of the NACA.

trade-named MultiHance,[82] which is albeit void of necrosis avidity. Indeed, it appears that both porphyrin and nonporphyrin NACAs exert their necrosis targeting function only when there exists denatured nonviable tissue debris in the living being, otherwise they just behave like other less specific CAs such as, for instance, ECF CAs used for the first-pass myocardial perfusion, blood pool CAs used for MR angiography, and hepatobiliary and urinary CAs for liver and kidney CE. NACA-induced strong adrenal CE also has been noticed in animal experiments.[42,74] Interestingly, porphyrins and an expanded porphyrin are reportedly able to target atherosclerotic plaques because of their preferential accumulation in the nonviable matrices of the plaques with or without uptake by macrophages.[30,83–85] Macrophage approach for plaque imaging has been documented with other particulate CAs.[86,87] Given the equivalent performances observed in studies on porphyrin and nonporphyrin NACAs, it is logical to expect that such an extra potential utility would apply to not only the complexes with multipyrrole rings but also other nonporphyrin NACAs. Further studies may reveal that plaque-targeting could well be one of the NACAs' versatile functions. Table 2 compares qualitatively some of the contrast enhancing properties between NACAs and other representative albumin binding blood pool, hepatobiliary, and ECF CAs.

INVESTIGATIONS OF THE MECHANISMS BEHIND NACAS

Regarding the mechanisms of NACAs, Hofmann et al[88] attributed specific accumulation of gadophrin-2 to its binding to albumin in the plasma and interstitium and subsequent trapping in intratumoral necrotic regions. However, this conclusion could not be proven in another study comparing gadophrin-2 and a strong albumin-binding blood pool CA MP2269.[89] This result suggests that only few albumin-binding CAs may possess the NACA property, although to some extent almost all NACAs tend to bind plasma proteins (typically albumin); in other words, the necrosis avidity is an outstanding feature beyond general pharmacokinetics of albumin-binding mediated drug transportation.

Hypothetically and partially supported by experimental observations, NACA-induced necrosis targeting may arise in a likely chemotactic fashion as follows.[89] While circulating in the blood pool after administration, the agents approach the necrotic region by a time-consuming process of perfusion through residual vessels, extravasation, and interstitial diffusion, wherein reperfused infarction is more favorable than occlusive infarction for NACA accumulation due to the ampler access. The disintegrated cell-membrane after autolysis facilitates contact and communication of NACAs with the tissue debris. After enzymatic denaturation, certain exposed radicles that are normally hidden inside intact macromolecules of cells and tissues may physicochemically attract and interact with a variety of internal and external chemicals to form strong bonds. Such interactions are usually indiscern-

TABLE 2. Comparison of the Functions Between NACAs and Control Agents

Properties	NACAs	MP-2269	Mn-DPDP	ECF CAs
First-pass perfusion	+	+	+	+
Hepatobiliary CE	+	+	+	−
Albumin-binding	+	+	?	−
Blood pool effect	+	+	−	−
Necrosis-avid CE	+	−	−	−
Plasma half-life*	2.0∼2.5 h	∼2.5 h	<0.5 h	<0.3 h

*From animal experiment.
+, effective; −, ineffective; ?, uncertain.

ible unless involving discernable labels such as dye, fluorescence, radioactive tracers, and magnetic metals. In the latter case, further augmentation of the relaxivity as the result of macromolecular interactions may in turn lead to a striking CE of the infarct on T1-weighted MRI[90] (Fig. 4). By ex vivo measurement, the T1 and T2 relaxivity of water protons with NACAs are typically close to 10 $(mM \cdot s)^{-1}$, which are more than twice of that with ECF CAs such as Gd-DTPA at about 4 $(mM \cdot s)^{-1}$.[6,34,39,45,57] However, once accumulated in necrosis, their relaxivity may be further unproportionally increased.[35,45]

Because of local high concentration of Gd resulting from such chemotactic accumulation, T2 and/or T2* susceptibility contrast enhancing effect can become predominant, especially on T2-weighted MRI (Fig. 4). Our recent studies suggest that such local interaction and retention seems strictly dependent on chemo-structure rather than a simple trapping or sluggish wash-in and washout because either a slight modification or even an isomer transformation may drastically switch off the necrosis-targeting effect of certain NACA molecules.[91,92] In respect to target tissues, the size and site of infarcted areas as well as the presence or absence of post-ischemic reperfusion determine what kind of NACA-induced necrosis-specific CE appears (ie, patchy or bulky, subendocardial or transmural, and complete or rim-like) and how long the CE may persist.[43] Unlike the "detrapping" process of nonspecific CAs over a few quarters of time,[8,34,45,75–77] the eventual clearance of NACAs from necrotic foci typically takes a few days after administration and parallels the natural healing process,[8,34,45] during which necrotic tissues are progressively infiltrated and phagocytized by inflammatory cells (mainly neutrophils, monocytes, and/or macrophages) and replaced by granulation tissues. Therefore, the retained NACAs in necrosis are most likely removed together with necrotic materials by phagocytosis. Thus, the secondary macrophage uptake after NACA-necrosis binding also may account for their local enrichment. Questions remain as for whether the Gd-complex of NACAs is still stable after being taken up by macrophages and what about the fate and consequence of this small necrosis-binding fraction of NACAs in the human body.[38,89] These details have to be further elucidated. Alternatively, to substitute the bio-incompatible lanthanide element Gd^{3+} with the physiological trace metal element Mn^{2+} in the complex of NACAs might eliminate the concerns about any potential side effects as the result of gadolinium body retention.[69,70]

EXPANDED SCOPE ON THE RESEARCH OF NACAS

Besides the aforementioned porphyrin and nonporphyrin NACAs, there appears to be a large variety of synthetic or natural, endogenous or exogenous substances, which all seem to share a common necrosis-avidity. These include the synthetic dye Evans Blue used for intravital staining,[93] the botanical extract hypericin derived from St. Johns Wort,[91,94,95] the heme-related cofactor hematoporphyrin for oxygen transportation,[16–19] and the urinarily excreted glucarate catabolized from UDP (uridine diphosphate)-glucose.[96,97] They all may firmly bind to the denatured nonviable tissue components, such as positively charged histone, collagen, and other reduced subcellular organelle proteins found in necrotic debris.[93–97] However, unless being inherently colored or fluorescent, their existence can hardly be discerned before their labeling with detectable markers as to form radio-[10–13,93–97] and magneto-pharmaceuticals.[6,8,38–49,51–69,88,89]

FROM FUNCTIONAL SIMILARITY TO STRUCTURAL DIVERSITY TO FINAL APPLICATIONS OF NACAS

The generally perceived structural diversity versus functional similarity, ie, the presence of porphyrin versus nonporphyrin, cyclic versus linear, natural versus artificial NACA-like chemicals,[91–97] supports our hypothesis that the avidity of certain chemicals to necrotic debris in the living body is an ever-existing phenomenon as part of the native wound healing process, which has never been well recognized yet deserves to be wisely exploited for medical purposes. To realize this goal, research gathering cross-disciplinary expertise is critically necessary. The key steps include understanding the underlying mechanisms of necrosis avidity and identifying the exact local configurations responsible for such strong physicochemical reactions through careful analyses on the structure–function relationship from all available NACA-like substances. Then, it might be possible to create dedicated all-in-one multifunctional CAs by purposely tailoring their chemical structures. Such molecular engineering might render additional NACA targetability onto any known substances, which could be derived from more physiological life molecules such as vitamins, amino acids and simple carbohydrates, as well as existing nontoxic medications already in use such as anti-ischemic and thrombolytic drugs or antineoplastic agents. This strategy may avoid hazards inherent with extreme artificial manipulations as exemplified to some degree by the development of "intelligent" CAs' consisting of totally nonphysiological substances.[98] The latter approaches are simply unrealistic for human applications and would ever remain investigational;[73,98] whereas the exploration utilizing natural processes may form a more operable, biocompatible, economical and ecological platform for research and development of CAs wherein more constructive interactions between academics and industries are supposed to be necessary and should be encouraged.

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