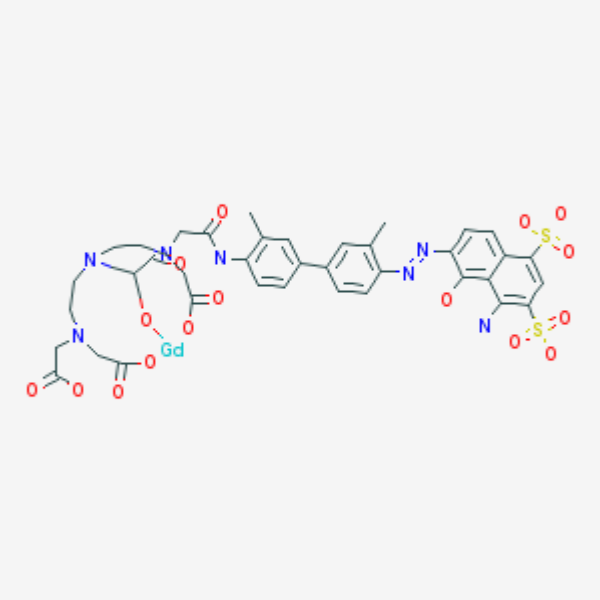
Evans Blue-diethylenetriamine-*N,N,N″,N″*-pentaacetic acid-gadolinium

EB-DTPA-Gd

Arvind Chopra, PhD[1]

Created: October 25, 2007; Updated: December 19, 2007.

Chemical name:	Evans Blue-diethylenetriamine-*N,N,N″,N″*-pentaacetic acid-gadolinium
Abbreviated name:	EB-DTPA-Gd
Synonym:	
Agent Category:	Compound
Target:	Vascular lesions
Target Category:	Nonspecific binding to proteins
Method of detection:	Magnetic resonance imaging (MRI)
Source of signal:	Gd
Activation:	No
Studies:	• *In vitro* • Rodents



Click on the above structure for additional information in PubChem.

Background

[PubMed]

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

NLM Citation: Chopra A. Evans Blue-diethylenetriamine-*N,N,N″,N″*-pentaacetic acid-gadolinium . 2007 Oct 25 [Updated 2007 Dec 19]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

Endothelium, the inner lining of blood vessels, is considered to be the largest organ of the animal body, and its major function, including the secretion of several bioactive molecules, is to maintain vascular homeostasis (1, 2). Any disruption of the normal epithelium functions or the formation of a lesion as a result of shear stress, pressure, cellular metabolism, or other unknown factors can trigger localized inflammation or the development of atherosclerotic plaques at the site of disruption (3). Therefore, early detection and determination of the degree of vascular injury is important for the development of a therapeutic regimen for this condition. Detection of the plaques in human coronary arteries is particularly important because, if left untreated, such plaques can result in myocardial infraction and even death. A variety of techniques, including computed tomography and magnetic resonance imaging (MRI), and different imaging agents linked to antibodies against proteins related to vascular disease have been designed and are now used to detect atherosclerotic plaques (4). However, most of these methods and agents are expensive and complicated to produce. Yamamoto et al. developed and evaluated an MRI contrast agent using a readily available and inexpensive dye, Evans Blue (EB), that could be used specifically to view vascular lesions (5). EB is a di-azo dye that has been used extensively for the determination of cell membrane permeability and blood volume in animals (6, 7). An MRI agent was generated by the conjugation of EB to diethylenetriamene pentaacetic acid (DTPA) to facilitate the binding of gadolinium (Gd) to obtain EB-DTPA-Gd, the contrast agent used in studies reviewed in this chapter (5, 8, 9).

Synthesis

[PubMed]

The synthesis of EB-DTPA-Gd was described by Yamamoto et al. (5). To start, *2,2*'-dimethylbenzidine (DMB) was dissolved in dichloromethane, and di-tertiary-butyl dicarbonate was added to it dropwise at room temperature with stirring. The reaction was allowed to proceed overnight, and excess DMB was removed with an aqueous saturated solution of tartaric acid. The organic phase was vacuum-concentrated, and the residue was purified with silica gel column chromatography to obtain N-tertiary butoxycarbonyl-DMB as a pale yellow solid. The yield of this reaction was 43%.

To synthesize the DTPA derivative of N-tertiary butoxycarbonyl-DMB, the two chemicals were dissolved in dimethylsulfoxide and the mixture was stirred at 40°C for 3 h. The resulting mixture was then poured into cold water, and the precipitate was collected by filtration. The precipitate was dissolved in sodium bicarbonate (pH 7.0) and purified with octadecylsilyl column chromatography. The appropriate fractions were pooled and freeze-dried to obtain a solid. The solid was dissolved in trifluoroacetic acid (TFA) and stirred at room temperature for 1 h. This solution was poured into cold ether, and the resulting precipitate was collected by filtration. The precipitate was then dried under vacuum to obtain DMB-DTPA 4TFA, a colorless solid. The yield of this reaction was 14%.

To obtain the DTPA derivative of EB, DMB-DTPA 4TFA was dissolved in water containing hydrochloric acid (HCl) and mixed with sodium nitrate for 20 min. This solution was then mixed for 3 h with sodium bicarbonate in a 1-amino-8-naphtol-2,4-disulfonic acid solution. The reaction mixture was freeze-dried, and the residue was dissolved in water. EB-DTPA was precipitated from the solution by mixing it with concentrated HCl. The yield of this reaction was 81%. The precipitate was collected and dried under vacuum.

To obtain the MRI contrast agent, equimolar solutions of gadolinium chloride ($GdCl_3$) and EB-DTPA were mixed. The pH of this solution was adjusted to 7.0 with sodium hydroxide. The solution was then lyophilized to obtain the contrast agent as a solid. Conditions for storage and stability of the contrast agent were not reported by the investigators (5).

The EB-DTPA used in some studies was synthesized as detailed above. Although DTPA-Gd was used in some studies, its synthesis or procurement from a commercial source was not reported by the investigators (5, 8, 9).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The use of EB-DTPA-Gd for the detection of vascular injury was evaluated *in vitro* with a porcine aorta section (5). The porcine aorta was opened flat, and the endothelium on the left half of the center axis was scraped with a scalpel while the right half was left intact. The entire aorta section was then dipped in a solution of EB-DTPA-Gd for 10 s and washed with saline. The aorta section was then evaluated with MRI. The T1-weighted MRI was reported to distinguish between the damaged and intact areas of the aorta, and MRI signal enhancement was observed only in the inner surface area of the aortic wall, indicating that the dye did not permeate into the tissue from the injured endothelium site (5). The MRI signal obtained with EB-DTPA-Gd was compared to that obtained with DTPA-Gd, and it was observed that the signal with DTPA-Gd was close to the background levels obtained with EB-DTPA-Gd. This was suggested to indicate that DTPA-Gd, compared to EB-DTPA-Gd, did not adsorb on the surface of the vascular lesion. The MRI signal was observed to increase with the concentration of EB-DTPA-Gd, and it was determined that a 10-mM concentration of EB-DTPA-Gd was sufficient to saturate the lesion area (5). At this concentration the MRI signal was 1.5 times that of the intact epithelium. It was also reported the signal could be obtained even in presence of serum (8).

Animal Studies

Rodents

[PubMed]

A balloon injury in the left carotid artery of rats was created as described elsewhere (10). The EB-DTPA-Gd contrast solution was then injected into the animals through the jugular vein, and T1-weighted MR images of the carotid artery were obtained through the transaxial plane (8). The investigators reported a clear increase in the signal from the injured artery compared to an intact common artery image in the control artery. In addition, an increase in MRI intensity was not observed when DTPA-Gd was used as the imaging agent either in the intact or the injured artery. This indicated that EB-DTPA-Gd could also be used in an *in vivo* setting (8). In the same study, the right (normal for use as a control) and left (injured by ballooning) common carotid arteries were extracted from the rats at 10, 30, and 120 min after reagent injection. The arteries were then opened and fixed on a glass plate for *ex vivo* MRI analysis as described above (5). The MRI signal intensity was highest at 10 min; it gradually decreased with time and was reported to be down to levels similar those of the intact artery at 120 min after the injection. With the results obtained from this study, the investigators concluded that EB-DTPA-Gd could be used for the non-invasive detection of vascular injury (8).

The use of EB-DTPA-Gd for the MRI detection of atherosclerotic lesions in apolipoprotein-E–deficient mice ($ApoE^{-/-}$ mice) was investigated by Yasuda et al. (9). $ApoE^{-/-}$ mice of different ages (3 to 12 months) were used in the study (n = 3–4 animals in each group), and age-matched normal mice were used as controls. The animals were intravenously injected with EB-DTPA-Gd through the tail vein, and MRI of the aorta of the animals was performed between 0 and 30 min after administration of the dye. The signal intensity was significantly ($P < 0.05$) different between the $ApoE^{-/-}$ (146 ± 33) and normal (62 ± 23) mice. In addition, an increase in intensity of the aortic wall signal was observed with an increase in age ($P = 0.05$). The investigators observed a correlation between the enhanced MRI signal and macroscopic location of the atherosclerotic lesion on the aorta as determined with Sudan Red staining (9). The investigators cautioned that, although the contrast agent appeared to be useful for non-invasive detection of atherosclerotic plaques, the *in vivo* bioactivity, specificity, and adverse effects of the agent remain to be investigated.

Other Non-Primate Mammals

[PubMed]

No publications are currently available.

Non-Human Primates

[PubMed]

No publications are currently available.

Human Studies

[PubMed]

No publications are currently available.

References

1. Hartge M.M., Unger T., Kintscher U. The endothelium and vascular inflammation in diabetes. Diab Vasc Dis Res. 2007;**4**(2):84–8. PubMed PMID: 17654441.
2. Tziomalos K., Athyros V.G., Karagiannis A., Mikhailidis D.P. Endothelial function, arterial stiffness and lipid lowering drugs. Expert Opin Ther Targets. 2007;**11**(9): 1143–60. PubMed PMID: 17845142.
3. Vanepps J.S., Vorp D.A. Mechano-pathobiology of atherogenesis: a review. J Surg Res. 2007;**142**(1):202–17. PubMed PMID: 17612564.
4. Briley-Saebo K.C., Mulder W.J., Mani V., Hyafil F., Amirbekian V., Aguinaldo J.G., Fisher E.A., Fayad Z.A. Magnetic resonance imaging of vulnerable atherosclerotic plaques: current imaging strategies and molecular imaging probes. J Magn Reson Imaging. 2007;**26**(3):460–79. PubMed PMID: 17729343.
5. Yamamoto T., Ikuta K., Oi K., Abe K., Uwatoku T., Murata M., Shigetani N., Yoshimitsu K., Shimokawa H., Katayama Y. First functionalized MRI contrast agent recognizing vascular lesions. Anal Sci. 2004;**20**(1):5–7. PubMed PMID: 14753250.
6. Hamer P.W., McGeachie J.M., Davies M.J., Grounds M.D. Evans Blue Dye as an in vivo marker of myofibre damage: optimising parameters for detecting initial myofibre membrane permeability. J Anat. 2002;**200**(Pt 1):69–79. PubMed PMID: 11837252.
7. Davy K.P., Seals D.R. Total blood volume in healthy young and older men. J Appl Physiol. 1994;**76**(5):2059–62. PubMed PMID: 8063668.
8. Yamamoto T., Ikuta K., Oi K., Abe K., Uwatoku T., Hyodo F., Murata M., Shigetani N., Yoshimitsu K., Shimokawa H., Utsumi H., Katayama Y. In vivo MR detection of vascular endothelial injury using a new class of MRI contrast agent. Bioorg Med Chem Lett. 2004;**14**(11):2787–90. PubMed PMID: 15125933.
9. Yasuda S., Ikuta K., Uwatoku T., Oi K., Abe K., Hyodo F., Yoshimitsu K., Sugimura K., Utsumi H., Katayama Y., Shimokawa H. In vivo Magnetic Resonance Imaging of Atherosclerotic Lesions with a Newly Developed Evans Blue-DTPA-Gadolinium Contrast Medium in Apolipoprotein-E-Deficient Mice. J Vasc Res. 2007;**45**(2):123–128. PubMed PMID: 17940339.
10. Uwatoku T., Shimokawa H., Abe K., Matsumoto Y., Hattori T., Oi K., Matsuda T., Kataoka K., Takeshita A. Application of nanoparticle technology for the prevention of restenosis after balloon injury in rats. Circ Res. 2003;**92**(7):e62–9. PubMed PMID: 12663484.