3,7-bis(dimethylamino)-phenothiazin-5-ium chloride

Methylene blue

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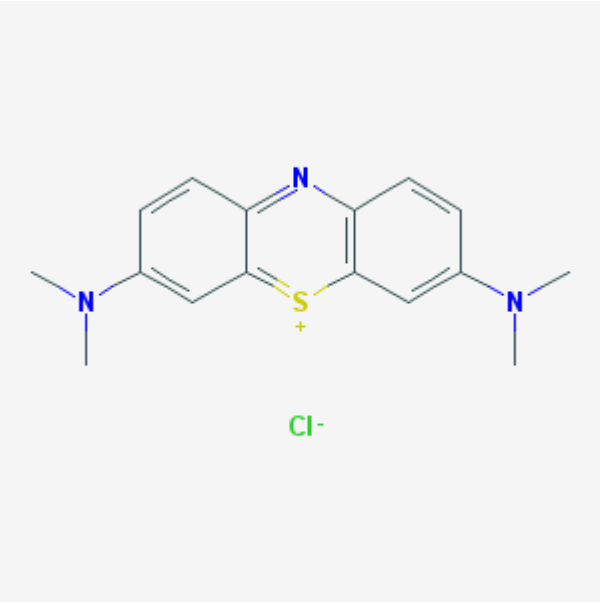
Chemical name:	3,7-bis(dimethylamino)-phenothiazin-5-ium chloride
Abbreviated name:	Methylene blue
Synonym:	[7-(dimethylamino)phenothiazin-3-ylidene]-dimethylazanium chloride
Agent Category:	Compound
Target:	Methemoglobin, guanylyl cyclase (putative), monoamine oxidase (putative)
Target Category:	Non-targeted for viewing synovial lymph nodes; Heme protein for methemoglobin
Method of detection:	Ultrasound imaging (photoacoustic imaging)
Source of signal / contrast:	Methylene blue
Activation:	No
Studies:	• *In vitro* • Rodents



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Background

[PubMed]

A sentinel lymph node (SLN)is the first lymph node showing presence of metastasized cancer cells from a tumor. An SLN biopsy (SLNB) is the method of choice to stage breast cancer (i.e., to describe the stage of cancer progress using numbers I to IV, going from low to highest progression) in patients exhibiting clinically negative (i.e., without metastases) axillary lymph nodes (1, 2). Once detected and confirmed, the metastatic spread of the cancer from the primary tumor to the axillary lymph nodes through the lymphatic system is then tracked with either a radiolabeled sulfur colloid and/or a blue dye (isosulfan blue or methylene blue; neither of these dyes are approved by the United States Food and Drug Administration (FDA) for this application) (2). After visual confirmation, the SLN is surgically resected, and the definitive presence of cancer is established with histological methods. Although the SLNB procedure is minimally invasive, it has a 5%–10% false negative rate of detecting cancer and can possibly lead to lymphedema, seroma formation (collection of fluid under the skin), injury to local sensory nerves, and a reduction in the patient's range of motion (1, 2). In some cases, ultrasonography (US), which is the detection of high frequency sound waves, is used to identify a SLN, and a biopsy is obtained with a fine-needle aspiration biopsy (FNAB). The biopsy sample is then subjected to molecular analysis using the real-time reverse transcription-polymerase chain reaction to detect cancer in the tissue from the breast. A limitation of using US to identify a SLN is that it can only determine the size and shape of the lymph node but cannot specifically distinguish between a cancerous and a non-cancerous lymph node; this limitation reduces the sensitivity of the FNAB procedure to detect a malignancy (3).

Photoacoustic imaging (PAI) is a noninvasive method that does not involve the use of ionizing radiation for the detection of cancer. This technique, which uses a combination of the optical and US imaging modalities to detect malignant lesions, angiogenesis, etc (4), has been described in detail elsewhere (5). In brief, application of a pulse of light to biological tissue results in the generation of ultrasonic waves (photoacoustic waves) due to the thermoelastic expansion and contraction of the tissue (i.e., a fraction of the light is converted into heat within the tissue). Subsequently, the photoacoustic waves are captured with a suitable device (transducer) that captures the US waves and converts them into an image. If used by itself, optical imaging has a limited imaging depth and spatial resolution because light is scattered by the tissue. As a result, images generated with US alone have a low contrast and are speckled (5). The main advantage of using PAI is that the depth and resolution of an image can be improved by controlling the detection frequency of the transducer, and the quality of the images can be further enhanced by using intrinsic contrast agents (e.g., melanin and hemoglobin) or exogenous contrast agents (e.g., an organic dye such as methylene blue, nanoparticles, etc.) (5).

Methylene blue is an aromatic compound that has many applications in biology and medicine. In the laboratory, methylene blue is often used to stain blood cells for histological investigations, to detect nucleic acids on blots, and to determine the viability

of yeast cells. Although methylene blue is not approved for clinical use by the FDA, it is available commercially in the United States for off label use as an intravenous injection to treat methemoglobinemia. Methylene blue is also under investigation in clinical trials to treat osteoarthritis, to detect invasive aspergillosis, and to map and localize SLN in breast and thyroid cancer patients. In several European countries, a procedure using methylene blue has been approved to reduce the pathogen load (e.g., viruses such as HIV and hepatitis B and C) in individual units of human plasma to be used for transfusion purposes (6). It has been shown that methylene blue radiolabeled with astatine-211 is suitable for the diagnosis and selective treatment of melanoma in mice (7). Investigators have also shown that methylene blue encapsulated in nanoparticles can be used for *in vivo* imaging and photodynamic therapy in mice (exposure of methylene blue nanoparticles to high-energy light pulses results in the production of nascent oxygen that damages the nucleic acids in the cells) (8).

Recently, in an effort to develop an alternative method to SLNB and FNAB for the detection of SLNs in breast cancer patients, methylene blue has been successfully used as a contrast agent to visualize SLN in rats with PAI (1, 2).

Other Sources of Information

Information on FDA website (methylene blue)

Other uses of methylene blue.

Synthesis

[PubMed]

The synthesis of methylene blue has been described in a patent (WO/2006/032879) available from the World Intellectual Property Organization (a specialized agency of the United Nations) (9). The dye is available in the United States as a 1% (W/V) solution in water for injection.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

In vitro kinetic assays and changes in visible spectra have shown that methylene blue is a potent reversible inhibitor of purified human monoamine oxidase (10). In a study to investigate the influence of methylene blue on rat hippocampal CA1 neurons, the dye was reported to decrease voltage-gated sodium channel currents and to modify the action potential of this tissue (11). However, it is important to mention these observations are not applicable to the visualization of SLNs because the dye appears to flow through the lymph system and does not localize in the nodes.

Animal Studies

Rodents

[PubMed]

The PAI system used to obtain the SLN images in rats consisted of a pulsed laser with a wavelength of 635 nm, which is close to 677 nm, the maximum absorption wavelength of methylene blue and ten-fold higher than that of 95% oxygenated hemoglobin (on a molar basis). The transducers used in conjunction with the pulsed laser had central frequencies of 3.5 and 5 MHz and focal lengths of 4.95 and 2.54 cm, respectively (1).

For the initial study, a rat was administered 0.07 ml 1% methylene blue in the left forepaw pad (1). PAI was performed on the animal immediately before and after injection. A SLN containing methylene blue was distinguished from a group of lymph nodes containing no dye as described by Song et al. (1). Without a methylene blue injection, only the vasculature around the lymph node was clearly visible with PAI (contrast 51 ± 7 compared to background). After the methylene blue treatment, the SLN was clearly visible (contrast 146 ± 41 compared to background), although the optical absorption of the blood also increased. After the administration of methylene blue, the contrast ratio between the SLN and the blood was reported to be ~2. The SLN was shown to be clearly visible for up to ~50 min after injection, even after repositioning the transducer. Similar results were obtained with PAI in another rat that had been injected with the dye. From this study, the investigators concluded that the PAI procedure was sensitive and suitable to detect methylene blue within a SLN in rodents (1).

In another study, a clinical US scanner was modified to perform PAI of SLN in rats ($n = 7$) injected with methylene blue as described above (2). From images obtained before and after injection in these animals, it was clear that SLN containing the dye generated strong photoacoustic signals, and the lymph nodes were visible up to 2.5 cm below the top surface of the overlying tissue. The animals were euthanized after the imaging was completed, and the presence of methylene blue in the SLN of the left axilla was confirmed with visual inspection of the lymph node. In addition, the optical spectrum of the photoacoustic signals was reported to match the optical absorption spectrum of methylene blue ($R = 0.995$) and confirmed the presence of the dye in the lymph nodes. The investigators concluded that this study was an important step toward the translation of PAI to the clinic for the noninvasive detection of SLNs (2).

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

Supplemental Information

[Disclaimers]

No information is currently available.

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