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Metilensko modrilo – lijek, bojilo, indikator

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Metilensko modrilo ili metiltioninijev klorid naziv je za 3,7-bis(dimetilamino)-fenotiazin-5-ijev klorid (slika 1.).

Slika 1. Strukturna formula metilenskog modrila.

Zajedno s azurom A, B i C pripada fenotiazinskim bojilima (razlikuju se po stupnju metilacije amino skupina). Prvi ga je sintetizirao Heinrich Caro 1876. godine. Od tada se koristi kao lijek, bojilo i indikator. Monografija o metilenskom modrilu nalazi se u većini farmakopeja, između ostalog u europskoj i američkoj farmakopeji (1, 2). U USP 37 su osim metilenskog modrila oficinalna i dva pripravka: Injekcija metilenskog modrila (slika 2.) i Injekcija metilenskog modrila za veterinarsku upotrebu. Metilensko modrilo se nalazi na listi esencijalnih lijekova Svjetske zdravstvene organizacije u skupini antidota i ostalih supstancija koje se koriste kod trovanja (3).

Slika 2. Otopina metilenskog modrila za intravensku primjenu.

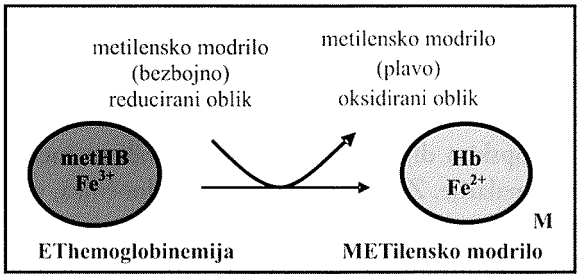
Metilensko modrilo je prvi potpuno sintetski spoj koji se koristio kao lijek. Paul Guttmann i Paul Erlich uveli su ga u terapiju malarije još 1891. godine. Do tada se kao antimalarik koristio kinin. Kinin se izolirao iz kore južnoameričke biljke kininovca (*Cinchona officinalis* L.), što je znatno ograničavalo opskrbu lijekom. Upravo je otkriće da jedna u potpunosti sintetska tvar, metilensko modrilo, djeluje protiv malarije, omogućilo bolje provođenje antimalarijske terapije (4). U Drugom Svjetskom ratu američki vojnici u tropskim dijelovima Pacifika dobivali su metilensko modrilo za prevenciju i liječenje malarije. Zbog bojanja urina i bjeloočnica, vojnici su ga uzimali nerado pa se ta praksa ukinula. No, u novije vrijeme interes za metilenskim modrilom kao antimalarikom ponovo raste (6–10). Sada je u tijeku nekoliko velikih kliničkih studija u kojima se ispituje antimalarijski učinak metilenskog modrila (11–13).

Danas se intenzivno proučava mogući utjecaj metilenskog modrila na razvoj Alzheimerove bolesti (14, 15). Naime, metilensko modrilo u *in vitro* uvjetima i na animalnim modelima inhibira agregaciju tau proteina i utječe na disocijaciju amiloida pa je pod zaštićenim imenima, prvo kao Rember, zatim pod nazivom LMTX ili TRx0237 ušao u klinička ispitivanja (trenutno je TRx0237 u trećoj fazi) kao potencijalni lijek za Alzheimerovu bolest (16). Najnovija istraživanja pokazuju da metilensko modrilo u malim dozama (0,5–4 mg kg^{-1}) poboljšava kratkotrajnu memoriju u ljudi (17), a *in vitro* istraživanja dodatno pokazuju učinke na usporavanje starenja stanica i sprječavanje disfunkcije mitohondrija, osobito enzima kompleks IV (18, 19). Također je dokazano njegovo neuroprotektivno djelovanje u eksperimentalnim modelima ishemičkog moždanog udara i Parkinsonove bolesti (20). Osim toga, metilensko modrilo modulira agregaciju intermedijera huntingtina pa pokazuje protektivni učinak u modelima još jedne neurodegenerativne bolesti – Huntingtonove bolesti (21).

Na američkim tržištu registrirano je nekoliko lijekova koji sadrže metilensko modrilo, a upotrebljavaju se u terapiji infekcija urinarnog trakta (22). Ti lijekovi sadrže kombinaciju antiseptika (metilensko modrilo, urotropin, benzojeva kiselina), analgetika i antiinflamatorika (fenil-salicilat) i spazmolitika (hiosciamin, atropin ili kombinacije alkaloida velebilja) (23).

Metilensko modrilo se može upotrijebiti kao antidot kod trovanja cijanidima, ugljikovim monoksidom (24), nitratima i nitritima (25), ali se u tu svrhu više ne upotrebljava. U farmakološkim dozama (1–2 mg kg^{-1}) djeluje kao reducens, a u višim dozama kao antioksidans. Upravo zbog reduktivnih svojstava koristi se u terapiji methemoglobinemije (MHb) uzrokovane nekim lijekovima ili toksinima (slika 3.). U našem organizmu methemoglobin se reducira u hemoglobin pomoću nekoliko methemoglobin reduktaza koje koriste NADH ili NADPH kao koenzime. Ako nastanu velike količine methemoglobina, kapaciteti enzima su nedovoljni pa je potrebno koristiti metilensko modrilo kao antidot (22). No, velike doze (20–30 mg kg^{-1}) metilenskog modrila induciraju methemoglobinemiju (26).

Metilensko modrilo inhibira enzim gvanilat ciklazu i monoamino oksidazu (MAO). Inhibicija prvog enzima sprječava nakupljanje cikličkog gvanozin monofosfata (cGMP)



Slika 3. Redukcija methemoglobina u prisutnosti metilenskog modrila.

zbog čega se smanjuje osjetljivost krvnih žila na cGMP-ovisne vazodilatatore kao što su dušikov(II) oksid i ugljikov(II) oksid (27). Zbog toga se metilensko modrilo može koristiti pri ekstremnoj hipotenziji uzrokovanoj sepsom (28) ili tijekom kirurških zahvata na srcu pri ugradnji premosnica (29). Inhibicija drugog enzima dovodi do serotoninskog sindroma ako se metilensko modrilo koristi intravenski u dozama većim od 5 mg/kg u kombinaciji sa selektivnim i neselektivnim inhibitorima ponovne pohrane serotonina (lijekovi iz skupine antidepresiva i anoreksika) (30). Zbog toga je kontraindicirana upotreba metilenskog modrila s tim lijekovima. Ne smije se koristiti niti uz MAO inhibitore (npr. uz antidepresiv tranilcipromin i moklobemid ili uz antiparkinsonike selegilin i razagilin).

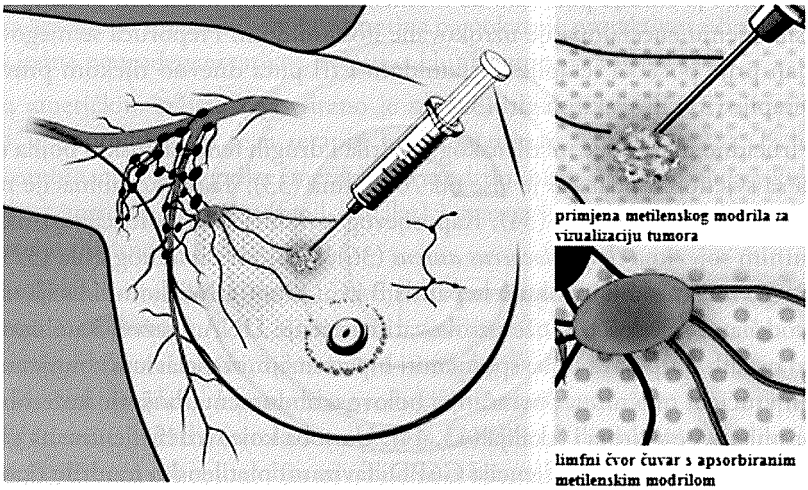
Nekoliko je studija pokazalo da metilensko modrilo može biti korisno u profilaksi i smanjenju encefalopatije uzrokovane ifosfamidom. Preporuča se njegova upotreba dan prije početka terapije ifosfamidom i tri puta dnevno tijekom provođenja kemoterapije (31) ili svakih 6 sati (32).

Virucidno djelovanje metilenskog modrila i drugih fenotiazinskih bojila u kombinaciji sa svjetlošću poznato je više od 70 godina (33). Takve kombinacije našle su primjenu u terapiji psorijaze (34), Kaposijevog sarkoma (35), infekcija uzrokovanih rezistentnim sojevima *Staphylococcus aureus* (36), virusom zapadnog Nila (37), HIV-om (38) te virusima koji uzrokuju hepatitis B i C (39, 40). No, kombinacija metilenskog modrila sa svjetlošću može uzrokovati oštećenje DNA i razvoj karcinoma (41, 42). Od ostalih nuspojava treba spomenuti hipertenziju, vrtoglavicu, zbunjenost, glavobolju, vrućicu, mučninu, povraćanje, bolove u abdomenu, bojanje kože i bjeloočnica, promjenu boje urina i fekalija (43, 44). U osoba koje su deficijentne na glukoza-6-fosfat dehidrogenazu (deficijencija G6PD, favizam) metilensko modrilo (kao i neki drugi lijekovi, npr. klorokin, primakin, sulfonamidi) uzrokuje hemolitičku anemiju (22). Naime, taj je enzim uključen u put pentoza-fosfata koji je posebno važan u crvenim krvnim stanicama. U literaturi je opisana i upotreba metilenskog modrila kao pomoćnog sredstva u terapiji vazoplegičnog sindroma nakon kiruškog zahvata na srcu (45, 46). Nekoliko pretkliničkih istraživanja ukazuju na selektivnu indukciju apoptoze stanica karcinoma nakon tretiranja metilenskim modrilom i srodnim fenotiazinskim bojilima (47, 48). Ispitivano je i djelovanje kombinacije metilenskog modrila i auksina u fotodinamskoj terapiji karcinoma (49).

Zanimljiva je i upotreba metilenskog modrila kao placeba. Pacijentima je sugerirano da je plava boja urina znak učinkovitosti terapije. U psihijatriji se pomoću metilenskog modrila kontrolirala suradljivost pacijenata te prihvaćanje režima liječenja. Zbog toga ne začuđuje činjenica da je upravo metilensko modrilo bilo vodeći spoj iz kojeg se razvio prvi neuroleptik klorpromazin (50).

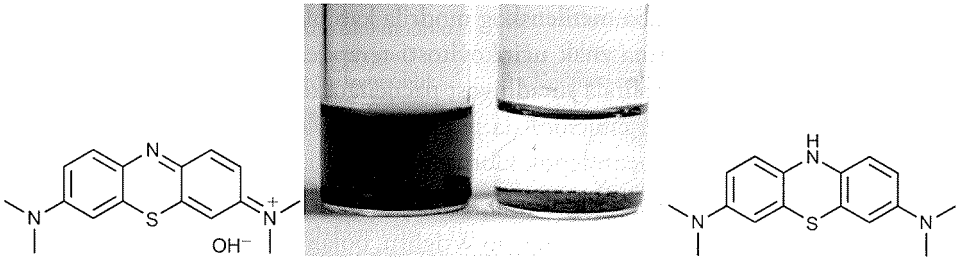
U biologiji se metilensko modrilo upotrebljava u nekoliko različitih postupaka za bojanje nukleinskih kiselina (bojanje po Wrightu, bojanje po Jenneru) (51). Koristi se i kao indikator preživljavanja stanica u testovima *in vitro*: žive stanice se ne oboje

jer se u njima metilensko modrilo reducira u bezbojnu formu, a mrtve stanice ostaju obojene (52). Važna je i njegova primjena u kirurgiji za označavanje tkiva na kojem će se obaviti manji kirurški zahvat (npr. odstranjivanje polipa) (53), za vizualizaciju limfnih putova pri odstranjivanju limfnih čvorova (slika 4.) (54) te u kemoendoskopiji za identificiranje displazije i prekanceroznih lezija u gastrointestinalnom traktu (55). U ortopedijskim operacijama dodaje se u koštani cement što omogućava lakše razlikovanje cementa od nativne kosti. Dodatno, ubrzava stvrdnjavanje koštanog cementa (56). Metilensko modrilo specifično boji živčana vlakna pa se na taj način proučavala inervacija mišića, kože i unutarnjih organa (slika 4.) (57).



Slika 4. Primjena metilenskog modrila za vizualizaciju limfnih čvorova (58).

Metilensko modrilo koristi se kao redoks indikator u analitičkoj kemiji (59): oksidirana forma metilenskog modrila je plava, a reducirana forma bezbojna (slika 5.). Nadalje, koristi se za fotometrijsko određivanje sulfida (60). Na tržištu je dostupan kit pod nazivom *Spectroquant Sulfide Test* za rutinsku analizu sulfida koji je npr.

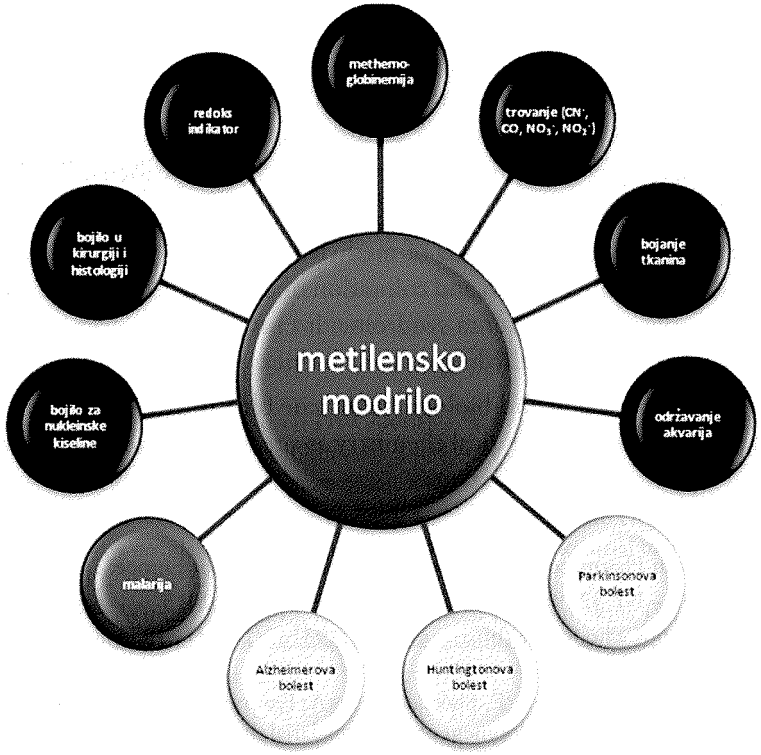


Slika 5. Oksidirana i reducirana forma metilenskog modrila.

pogodan za brzu detekciju bakterija koje reduciraju sulfate (61). Metilensko modrilo se koristi i u tzv. MBAS testu (*Methylene Blue Active Substances Assay*), kojim se dokazuje prisutnost anionskih surfaktanata u vodi (62). Metilenskim testom može se odrediti i kapacitet gline za apsorpciju kationa iz otopine. Za tu svrhu proizveden je kit pod nazivom *Methylene Blue Kit* (63). U sintetskoj organskoj kemiji metilensko modrilo može se koristiti za sintezu peroksida. Reakcija ide preko singletnog kisika koji nastaje reakcijom metilenskog modrila s kisikom u prisutnosti svjetlosti (64).

Metilensko modrilo ima i komercijalnu primjenu: koristi se u akvarijima za tretman i prevenciju infekcija gljivicama i bakterijama (65) te u tekstilnoj industriji kao boja za tkanine.

Sumarno, na slici 6 prikazana su područja u kojima se upotrebljavao, upotrebljava ili će se moguće upotrebljavati metilensko modrilo.



Slika 6. Prijašnja, sadašnja i potencijalna upotreba metilenskog modrila.

Methylene blue - medicine, dye, indicator

B. Zorc, K. Pavić

Abstract

Methylene blue is a trivial name of 3,7-bis(dimethylamino)-phenothiazine-5-ium chloride). It has many uses in medicine, pharmacy, biology and chemistry. It is on the World Health Organization's List of Essential Medicines. From the end of 19th century to the end of Second World War methylene blue was used in therapy and prevention of malaria. Interest in its use as an antimalarial agent has recently been revived and several clinical trials are in progress. Another clinical trials investigate usefulness of methylene chloride for treatment of Alzheimer's dementia, ischemic stroke, Parkinson and Huntington's diseases.

Several papers report that methylene blue and related phenotiazine dyes appear to induce selective cancer cell apoptosis. Combined with light it has virucidal properties as well and combined with light and auxin it has a potential for the photodynamic treatment of cancer. Intravenous injections of methylene blue are used for the treatment of methemoglobinemia and as antidote in cyanide, carbon monoxide, nitrate and nitrite poisoning. It is used before initiation and during ifosfamide chemotherapy for treatment and prevention of ifosfamide neurotoxicity. In combinaton with other drugs it is used in treatment of urinary infection.

The most important side effects of methylene blue are hypertension, dizziness, headache, nausea, staining of skin and coloration of urine. Taken together with MAO inhibitors may precipitate serotonin syndrome. It causes hemolytic anemia in carriers of the glucose-6-phosphate dehydrogenase deficiency.

In surgery, endoscopy and biology methylene blue is used as a dye in different staining procedures and in analytical chemistry as a redox indicator, in sulfide analysis and in detection of anionic surfactants.

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