

Critical Relation Between Methylene Blue and Red Light in Anti-Cancer Activity: A Comprehensive Review

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ABSTRACT

Photodynamic therapy (PDT) using MethyleneBlue (MB) activated by red light has emerged as a promising anticancer approach owing to its selectivity, low cost, and minimal systemic toxicity. Upon activation with red light (630–660 nm), MB generates Reactive Oxygen Species (ROS), leading to oxidative damage and cancer cell death. Evidence from multiple in vitro and preclinical studies demonstrates that MB-PDT effectively induces cytotoxicity in cancer cells, especially in breast cancer cells, while sparing normal cells. This review critically examines the photochemical basis, molecular mechanisms, experimental findings, therapeutic advantages, and future prospects of MB-mediated red-light therapy in oncology.

KEYWORDS: Methylene blue (MB), Photodynamic therapy (PDT), Red light therapy, Anticancer activity, Reactive oxygen species (ROS), Photosensitizer.

I. INTRODUCTION

Cancer treatment faces major challenges, including drug resistance, recurrence, and non-selective toxicity. Photodynamic therapy (PDT) has gained attention as a targeted alternative that combines.

- A photosensitizing agent
- Light of a specific wavelength
- Molecular oxygen

Methylene blue (MB), a well-known phenothiazine dye, is increasingly being studied as a photosensitizer because of its favourable photophysical properties. When activated by red light, MB produces cytotoxic species that selectively eliminate cancer cells¹.

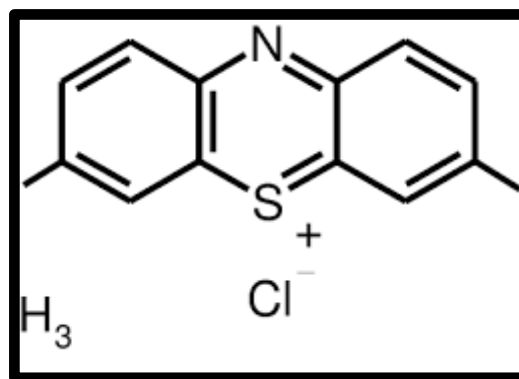


Fig.1 Chemical structure of Methylene Blue

II. Photophysical and Chemical Basis of MB-PDT

2.1 ABSORPTION AND ACTIVATION

- MB strongly absorbs light in the red region (~660 nm), which allows for the following:
- Deeper tissue penetration compared to shorter wavelengths
- Efficient activation in biological tissues

Upon irradiation:

- MB transitions to an excited singlet state
- Converts to a triplet state
- Transfers energy to oxygen, generating ROS

2.2 REACTIVE OXYGEN SPECIES GENERATION

The primary cytotoxic agents include:

- Singlet oxygen
- Superoxide radicals
- Hydroxyl radicals

ROS are responsible for oxidative stress-induced cell death².

III. Mechanisms of Anticancer Activity

3.1 OXIDATIVE DAMAGE

ROS generated by the MB-PDT attack.

- Lipid membranes → permeability loss

- Proteins → structural and enzymatic dysfunction
- DNA → strand breaks and mutations

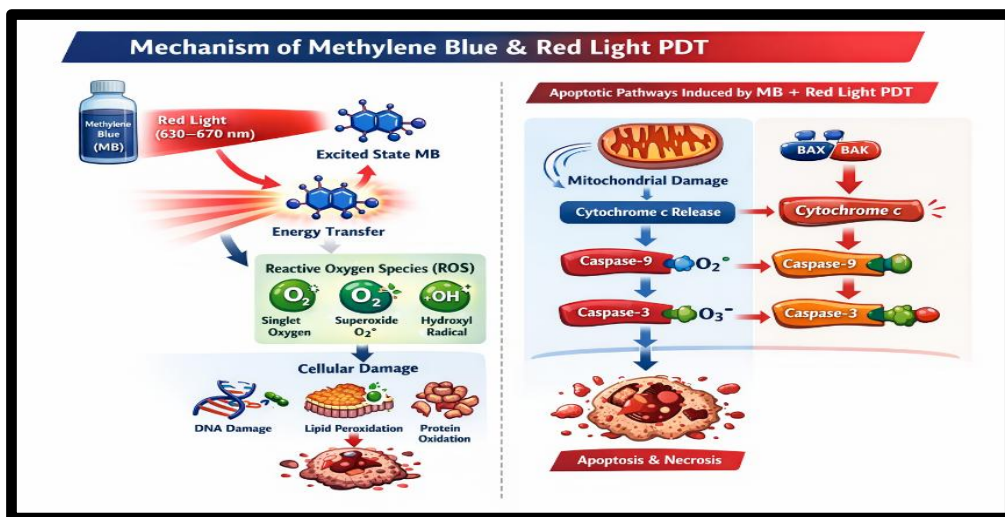


Fig.2- Mechanism of action of Methylene Blue and Red Light.

3.2 ORGANELLE-SPECIFIC EFFECTS

MB localizes preferentially in the

- Mitochondria → disruption of ATP production
- Lysosomes → membrane permeabilization

This leads to catastrophic intracellular damage.

3.3 NON-CLASSICAL CELL DEATH PATHWAYS

Unlike chemotherapy, MB-PDT often induces

- Necrotic cell death
- Autophagy-associated death
- Lysosome-mediated pathways

Notably, apoptosis is not always the dominant mechanism, allowing MB-PDT to bypass the apoptosis resistance in tumours³.

IV. Selective Cytotoxicity Toward Cancer Cells

4.1 EVIDENCE FROM BREAST CANCER STUDIES

A widely cited experimental study using breast cell lines showed that:

- Triple-negative breast cancer cells: ~98.6% cell death
- Luminal breast cancer cells: ~93% cell death
- Normal breast epithelial cells: ~52% cell death

This demonstrates preferential toxicity toward malignant cells, particularly aggressive ones subtypes⁴.

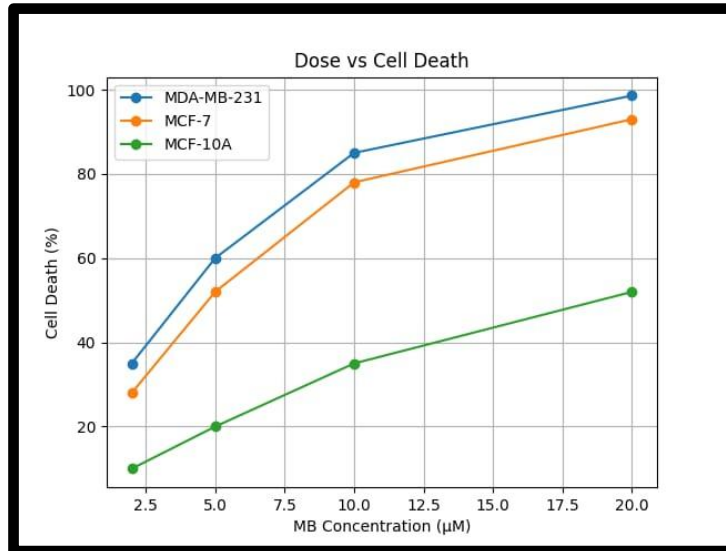


Fig.3 – Relation between Dose and Cell Death.

4.2 BIOLOGICAL BASIS OF SELECTIVITY

The selectivity arises from the following:

a. Elevated Baseline ROS

- Cancer cells operate under oxidative stress, making them more vulnerable to ROS overload.

b. Impaired Antioxidant Systems

- A reduced ability to neutralize ROS leads to rapid cell damage.

c. Increased MB Uptake

- Altered membrane transport in tumor cells enhances the accumulation of dyes⁵.

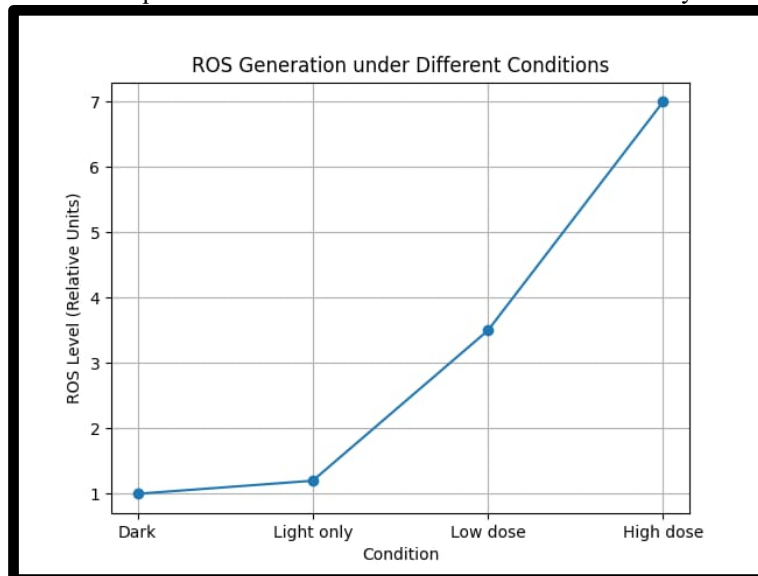


Fig.4- Graph showing variation at different values of ROS.

V. Experimental Evidence Across Cancer

Types

A systematic review of preclinical studies (2023) reported that

- MB-PDT reduced tumour size in multiple cancer models
- Effective across carcinoma, melanoma, and colorectal cancer

- Enhanced efficacy when combined with nanocarriers

In addition:

- Nanoparticle-based delivery improves cellular uptake and ROS generation

- Liposomal MB systems enhance intracellular delivery and therapeutic efficiency⁶.

VI. Treatment Parameters Influencing Efficacy⁽⁷⁾

| Parameters | Data/Result |
|------------------|--|
| Light Parameter | 1.Wavelength: - 630-660nm 2. Energy dose: - 4-10J/cm ² |
| MB Concentration | 1.In Vitro ~2–20 μM 2.In Vivo-0.04–24.12 mg/kg |

VII. Advantages of MB-Red Light Therapy

7.1 HIGH SELECTIVITY

The preferential destruction of cancer cells reduces collateral damage.

7.2 MINIMAL SYSTEMIC TOXICITY

MB exhibits low toxicity in the absence of light activation.

7.3 COST-EFFECTIVENESS

Compared with advanced biologics, MB is inexpensive and widely available⁸.

7.4 VERSATILITY

Can be combined with:

- Surgery (to eliminate residual cells)
- Chemotherapy
- Radiotherapy

VIII. Limitations and Challenges

8.1 LIMITED LIGHT PENETRATION

Red light penetrates only a few millimetres, restricting the treatment of deep tumours.

8.2 TUMOUR HYPOXIA

Low oxygen levels in tumours reduce ROS generation and therapy efficacy.

8.3 LACK OF CLINICAL TRIALS

Most studies are:

- In vitro
- Animal-based

However, clinical translation remains limited.

8.4 DRUG DELIVERY ISSUES

Free MB may show:

- Poor cellular uptake
- Rapid clearance

Whereas changes to such issues can yield better result⁹.

IX. Recent Advances and Innovations

9.1 NANOTECHNOLOGY INTEGRATION

- Liposomes and nanoparticles improve targeting
- Enhance intracellular accumulation

9.2 COMBINATION THERAPIES

- MB-PDT + immunotherapy
- MB-PDT + chemotherapy

9.3 IMPROVED LIGHT DELIVERY

- Fiber optics
- Implantable LEDs

These innovations aim to overcome the depth and targeting limitations¹⁰.

X. Future Perspectives

Future research should focus on the following aspects:

- Large-scale clinical trials
- Optimization of dosing protocols
- Targeted delivery systems
- Overcoming tumour hypoxia
- MB-PDT also shows potential in the following areas:
 - Drug-resistant cancers
 - Triple-negative breast cancer

XI. CONCLUSION

The critical relationship between methylene blue (MB) and red light represents a highly promising frontier in photodynamic therapy (PDT) for oncology. Upon targeted irradiation with red light, MB functions as a potent photosensitizer, driving the localized generation of lethal reactive oxygen species (ROS). This synergistic activation effectively induces selective cytotoxicity, bypassing

traditional apoptotic resistance mechanisms through alternative cell death pathways while largely sparing surrounding healthy tissue. Furthermore, MB's inherent affinity for mitochondria and lysosomes amplifies intracellular oxidative damage, cementing its efficacy at the cellular level.

To fully harness the therapeutic potential of the MB-red light axis, future research must prioritize the development of advanced pharmaceutical formulations. Leveraging engineered polymer matrices and novel excipients to refine MB drug delivery and controlled release profiles will be crucial for enhancing targeted tumor accumulation and overcoming physiological barriers. Ultimately, while the phototoxic efficacy of MB activated by red light is unequivocally established, ongoing interdisciplinary innovations in formulation science, nanotechnology, and light-delivery systems are essential to elevate this targeted modality from experimental promise to standard clinical practice.

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