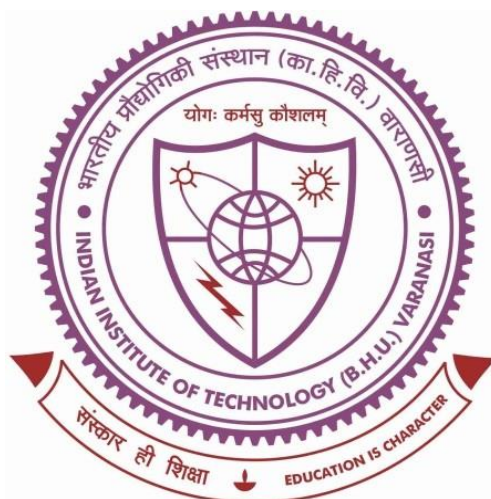


DIMETHYL FUMARATE AND ITS COCRYSTALS: POTENTIAL BIOLOGICAL APPLICATIONS



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By

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Chapter 5
Summary & Conclusion

5 Summary & Conclusion

Multiple sclerosis (MS) is an auto-immune disorder in which the T lymphocytes play a crucial role. T lymphocytes like CD8⁺ and CD4⁺ T cells contain cytotoxic granular enzymes like cathepsin C and granzyme B which are implicated in MS. Dimethyl fumarate (DMF) is an ester-based oral drug used for the management of relapsing-remitting multiple sclerosis (RRMS). The reported mechanism of action is related to antioxidant only which is a generalised mechanism and not all antioxidant drugs have immunomodulatory activity. Therefore, we have explored an immunological target-cathepsin C in the EAE model of MS. Moreover, DMF has severe adverse effects such as diarrhoea, dyspepsia, irritable bowel syndrome, erosive gastritis, gastric ulcer and gastroduodenitis, lymphopenia, and progressive multifocal leukoencephalopathy. Further, DMF has a serious physicochemical problem of sublimation due to which about 20% is lost from the bulk during storage and conventional manufacturing process. In order to solve these issues, we have explored the mechanism of action of DMF on a novel target contained in the T lymphocytes- cathepsin C and prepared various cocrystals of DMF to overcome its sublimation-related physical stability problems and gastric adverse effects.

In our experiment, we observed that DMF pharmacological activity also includes targeting lymphocytes and exerting its immunomodulatory activity by inactivating an important upstream target (cathepsin C), and controlling essential apoptotic proteins like granzyme B. The proven therapeutic effectiveness of DMF in the treatment of various autoimmune diseases is likely due to the irreversible inhibition of prominent enzyme cathepsin C followed by granzyme B inactivation, thus impacting several cellular events involved in immune activation. For the first time, we have found the effect of DMF on enzymatic activity and kinetics of cathepsin C in an *in-vitro* experiment. Moreover, we

have evaluated the effect of DMF on the activity of cathepsin C and granzyme B in the experimental autoimmune encephalitis (EAE) model of MS. DMF has been shown to ameliorate the disease-induced clinical symptoms and demyelination of the spinal cord showing the efficacy of DMF in EAE model of MS. Moreover, DMF has reduced the CD8⁺ and CD4⁺ T cells infiltration into the brain and spinal cord. Therefore, this research could provide insight into understanding the probable mechanism of action of DMF in the treatment of RMMS.

We have prepared a novel cocrystal of DMF with nicotinamide as a coformer based on their predictability to participate in hydrogen bonding and GRAS (generally regarded as safe). The cocrystals have been formulated by using the solvent evaporation method and characterized using spectral techniques of FTIR and diffractometry techniques of PXRD. The thermal evaluation has been done using TGA and DSC. The physical test was performed to evaluate the sublimation rate of DMF and its cocrystal. Dissolution and pharmacokinetic studies have been conducted to compare the release profile of DMF with its cocrystal in *in-vitro* and *in-vivo* systems. The cytotoxic and biological activity of DMF has been compared with that of the cocrystal. DMF-NIC cocrystal has shown protection against gastric ulcers as compared to DMF. For the very first time, DMF cocrystals have been made to troubleshoot its sublimation problem and also to counterbalance its adverse effects. This will ultimately lead to enhanced processability of the API during its formulation and also improved safety with minimal side effects.

Another two cocrystals of DMF have been prepared using citric acid and succinic acid as coformers which overcomes the sublimation-related physical stability problem of DMF. These cocrystals have been formulated by using the solvent evaporation method and were characterized by FTIR and PXRD. The thermal evaluation of cocrystals has been done

with the help of TGA, DSC and physical stability test. The T_{onset} and melting point of the cocrystals have been enhanced as compared to DMF. This shows an improvement in its thermal stability. Dissolution and pharmacokinetic studies compared the release profile and absorption, distribution, metabolism and excretion (ADME) of DMF with its cocrystal in *in-vitro* and *in-vivo* systems. The cocrystals were non-toxic with better biological activity. Therefore, cocrystals can be used as a potential tool to modulate the physicochemical character and biological activity of DMF.

5.1 Important outcomes

- ❖ DMF acts on the CD4+ and CD8+ T lymphocytes by irreversibly inhibiting the cathepsin C enzyme.
- ❖ DMF indirectly inhibits granzyme B as it is the downstream enzyme controlled by cathepsin C.
- ❖ DMF ameliorates the infiltration of CD4+/CD8+ T lymphocytes in the CNS.
- ❖ DMF ameliorates demyelination of the spinal cord.
- ❖ Cocrystals of DMF have shown similar pharmacokinetics as that of neat DMF.
- ❖ DMF-NIC cocrystals have shown thermal stability and gastroprotective activity with better biological activities than DMF alone.
- ❖ DMF-SUCC and DMF-CIT cocrystals have shown better thermal stability and biological activities than DMF.

5.2 Future studies

- ❖ DMF treatment has reduced the CD4+ and CD8+ T lymphocyte counts, the mechanism involved and the role of cathepsin C can be further explored.
- ❖ The effect of cocrystals of DMF on cathepsin C can be evaluated.

- ❖ The effect of cocrystals of DMF on granzyme and demyelination can be evaluated.
- ❖ Single crystal XRD of the cocrystals can be performed to have a better view of the crystal lattice of the cocrystals.

5.3 Impact on the treatment of multiple sclerosis

DMF and its metabolite MMF exhibited irreversible inhibition of cathepsin C which is a crucial enzyme involved in the apoptosis of neurons and oligodendrocytes. DMF has ameliorated EAE by inhibiting cathepsin C and granzyme B. A novel immunological mechanism of action of DMF has been found and reported which can be implicated in the treatment of multiple sclerosis. Moreover, cocrystals of DMF have shown better thermal stability, lesser adverse effects and better biological activities without affecting the pharmacokinetic profile of DMF which can help in formulation stability and formulation development of DMF.

