CONCLUSION
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Limited availability of chemotherapeutics against brain tumour is a major concern in chemotherapy of brain tumour. As many of the chemotherapy are not able to cross BBB extending application of existing chemotherapeutics is a better approach than finding new chemotherapeutics. Methotrexate with a long history of efficacy against different type of cancer is an ideal candidate for enhancement of application against brain tumour. Investigations to overcome BBB for MTX have so far not led to its successful application. To provide an alternative and enhance brain availability of MTX we adopted the carried mediated transportation approach.

Amino acids are essential for normal functioning of brain. Being polar in nature they cannot cross BBB passively and depend on active transport system present at BBB for their brain availability. So developing drug conjugates which have similar features to amino acids is a novel approach, which has been shown in some cases to enhance brain availability. As there are many amino acid transporters with good range of substrate flexibility including ATB0,+ , PEPT1, LAT1, LAT2 it was considered worthwhile to develop their conjugates with MTX for enhancing its brain availability. Reversible conjugates of MTX and amino acids were developed in order to regenerate MTX at the target site (Brain). The ease of hydrolysis and peripheral stability are two factors which are generally crucial to the success of this kind of conjugates. So both ester and amide conjugate of amino acids were developed and analyzed.

Anionic amino acid like L-glutamate is transported to brain mediated by sodium independent x’ transporter system. This transporter has low substrate capacity. However its amide i.e. glutamine is a good substrate of LAT1 transporter which has high substrate capacity as well as higher substrate flexibility. So MTX-GLU was developed keeping in view the structural features of glutamine. The conjugate was found to be stable enough for optimum peripheral stability. It was also shown to hydrolyze slowly in brain homogenate suggesting slow release of MTX which is desirable for chemotherapy of cancer. Following intravenous administration the relative uptake efficiency was 5.76 with a concentration efficiency of 5.28. This suggested significant brain availability of MTX from the conjugate without any impact on toxicity profile of MTX.

Cationic amino acid L-lysine cannot be synthesized in brain and hence taken up from circulation for optimum brain function facilitated by transporter system γ+ and LAT1 at BBB. Based on this a novel lysine conjugate of MTX was developed which was shown to increase
the brain penetration of MTX. The chemical stability of MTX-LYS was sufficient for i.v. administration. Its protein binding was comparable to the parent drug, but it was more polar which is supposed to limit its passive permeation. It was stable enough in the plasma to survive for brain transportation. The enzymatic release of MTX from MTX-LYS was slower both in vivo and in vitro, which can be an essential feature for sustained drug action. The peripheral tissue distribution of MTX-LYS was less but brain transport was more compared to MTX, which provided evidence of its suitability for selective brain transport.

Neutral amino acids including phenylalanine, leucine and tyrosine in the plasma are transported into the brain across the blood–brain barrier by LAT1 at BBB. These amino acids show higher affinity for this system expressed at BBB than those expressed peripherally. Capitalizing on this Tyrosine conjugate of MTX, MTX-TYR was shown to increase the brain penetration of MTX. The stability study of MTX-Tyr suggested its suitability for i.v administration and stability in the blood circulation for brain transportation. The peripheral tissue distribution of MTX from MTX-TYR was less but brain transport was more compared to MTX administered alone. This suggested selectivity for brain transport. Toxic profile of the conjugate was similar to that of MTX. However upon repeated administration it shows some toxicity which is usually associated with chemotherapy. This can be attributed to the enhanced availability of MTX in brain. In continuation of this approach we developed phenylalanine (MTX-PAL) and leucine (MTX-LEU) conjugate of MTX. Both conjugates were found to have good permeability. However the relative uptake efficiency of MTX-LEU was higher than that of the MTX-PAL. Both conjugates were shown to release MTX slowly in brain with good peripheral stability. There was no significant effect on toxicity as compared to MTX. Peripheral tissue distributions of conjugates were found to be less compared to brain distribution suggesting some selectivity in MTX brain delivery.

These studies substantiate the utility of amino acids for use as carrier for enhancing brain availability of MTX. Although the exact affinity of these conjugate to the transporters is not clear in the study, enhancement of brain availability by different type of amino acids indicates involvement of multiple transporters. As these transporters are also expressed in the tumour, these conjugates also can have potential for enhanced tumour uptake which is a desirable in chemotherapy and may help in overcoming resistance. Although it requires further investigations to materialize these possibilities, at present these conjugates provide an
viable opportunity for enhancing brain availability of MTX and thereby enhance the scope of brain tumour chemotherapy with MTX.