CEREBRAOPROTECTIVE EFFECTS OF INDOLE-3 CARBINOL AND ITS MAJOR METABOLITE IN ISCHEMIC MODEL

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Doctor of Philosophy

By

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Chapter 6

Summary & Conclusion

6 Summary and conclusion

The important findings are that I3C and DIM treatments exhibit the neuroprotection by ameliorating the platelet aggregation, thrombus generation, oxidative stress, inflammation, and stimulating the mitochondrial biogenesis.

The pharmacokinetics of I3C between sham and MCAO rats did not alter, but brain penetration was higher in MCAO, possibly due to a compromised BBB. DIM levels were elevated after oral administration of I3C than i.v. administration. Pharmacodynamic studies showed that oral administration of I3C ameliorated the MCAO-induced neurological deficits, brain infarction, BBB leakage, and brain edema more than parenteral administration. Therefore, the better pharmacological activity of I3C in the oral route may be due to high levels of DIM. These findings retrieve that DIM may be mediating the pharmacological actions of I3C.

Based on the above findings, we believe that DIM may mediate the pharmacological effects of I3C. Further, *in silico* studies predicted that the indole group of DIM and the hydroxyl group of I3C are responsible for modulating platelet interaction with the GPVI and P_2Y_{12} receptors. DIM has a stronger interaction with GPVI and P_2Y_{12} receptors than its parent compound, I3C. *In vitro* studies indicate that DIM more potently inhibited ADP, collagen, thrombin, and arachidonic acid-induced platelet aggregation and thrombin-induced clot retraction than I3C. *In vivo* results from $FeCl_3$ induced carotid artery thrombosis indicated that DIM prolonged the TTO and reduced the clot weight. This could be due to increased cAMP and inhibition of platelet oxidative stress and COX-1 enzyme-mediated biosynthesis of TXB_2 and PGE_2. Further, DIM prolonged the bleeding and clotting time than I3C. However, DIM and I3C did not cause thrombolysis. These findings confirm that DIM is

a more potent inhibitor of platelet aggregation and thrombus generation than I3C and DIM mediated the antiplatelet and antithrombotic activities of I3C.

We further assumed that the aforementioned *in vitro* and *in vivo* findings of DIM might be beneficial for mitigating stroke. Accordingly, DIM improved the neurobehavioral outcomes in focal cerebral ischemic injured rats. DIM confers the neuroprotection could be due to the amelioration of platelet aggregation, oxidative stress, and inflammation in MCAO rats. The higher dose of DIM (50 mg/kg) shows equipotent amelioration of MCAO-induced abnormalities with CLOP treatment. These findings retrieved that DIM is beneficial for the management of stroke.

Peripherally, I3C inhibited platelet aggregation and thrombosis in MCAO rats. Moreover, according to our pharmacokinetic data, we found high amounts of I3C in the brains of MCAO rats. Therefore, we predicted that I3C could have central protective mechanisms. Centrally, I3C treatment ameliorated neurological dysfunction by preserving mitochondrial function in focal cerebral ischemic rats. Repeated administration of I3C significantly alleviated the MCAO-induced sensory-motor dysfunction, brain infarction, BBB leakage, brain edema, oxidative stress, and inflammation. Further, I3C improved the mitochondrial function in stroke by activating the AMPK/PGC-1α pathway and its downregulated proteins, including NRF1, NRF2, and tFAM, thereby protecting the neurons from ischemic injury. Therefore, the present investigations conclude that I3C and DIM are possible candidates as antiplatelet, antithrombotic, and neuroprotective for treating ischemic stroke.

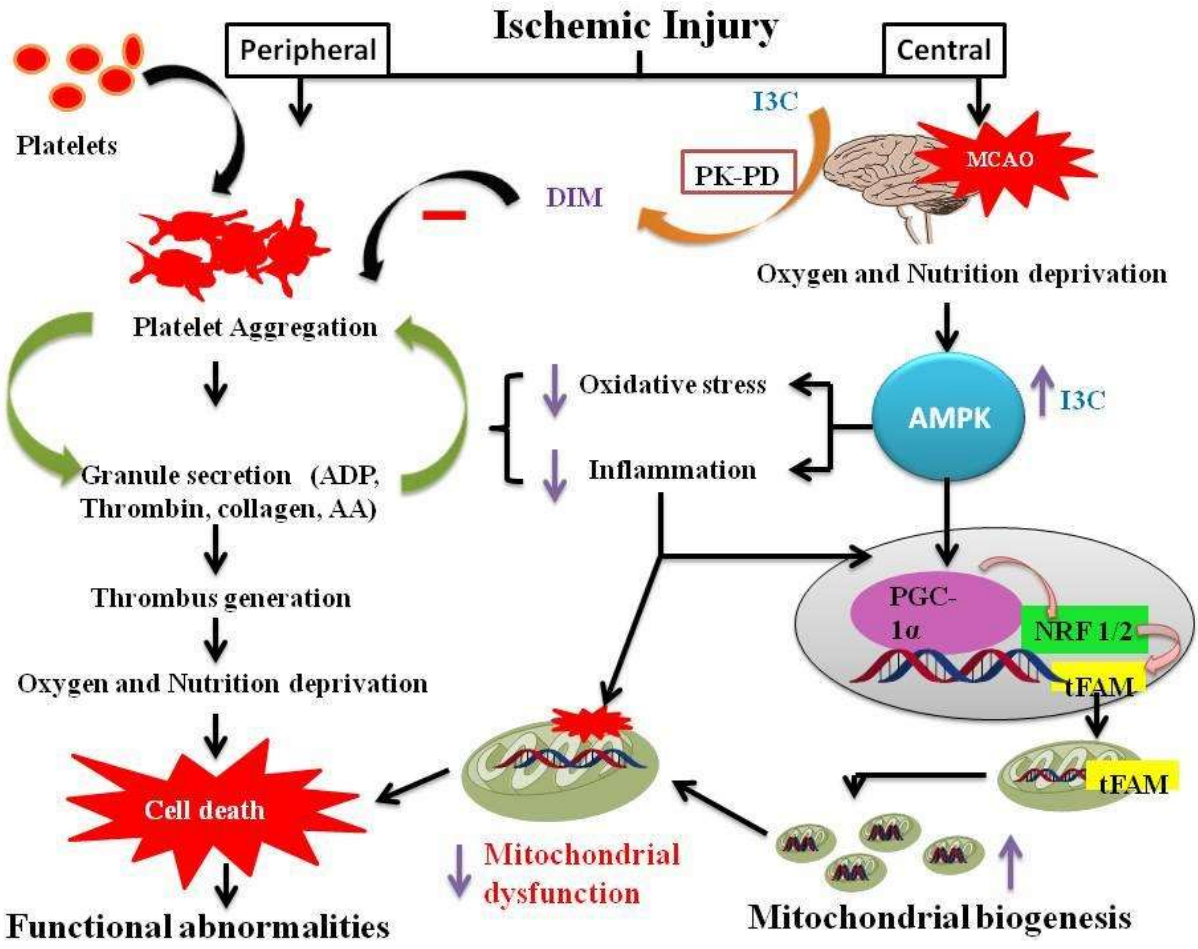

Figure 6.1 Summary and conclusion of the study: blue arrow (downwards) represents decrease; blue arrow (upwards) represents decrease; denotes increase, ▬ symbol indicates the inhibition.

6.1 Important outcomes

- I3C pharmacokinetics did not alter in pathological conditions of stroke, but brain penetration of I3C and DIM raised in cerebral ischemia. The oral administration of I3C generated higher amounts of DIM and I3C given orally was more beneficial than I3C given intravenously in the treatment of stroke.

- I3C major metabolite, DIM, exhibits antiplatelet and antithrombotic activity but did not show thrombolytic activity.

- DIM antiplatelet, antithrombotic, antioxidant, and anti-inflammatory activities are responsible for neuroprotection against MCAO-induced ischemic brain injury.
- Repeated I3C treatment stimulates mitochondrial biogenesis and mitigates the MCAO injury in rats.

6.2 Future studies

- We found that DIM oral treatment exhibited antiplatelet and antithrombotic activities. However, pharmacological effects of intravenous of DIM therapy has to be investigated.
- Investigation of DIM pharmacokinetics would help to rationalize dosing regimens for treating stroke.
- We found that I3C improved mitochondrial function in MCAO rats. However, DIM role in protecting the mitochondria in MCAO rats has to be evaluated.
- Antiplatelet agents like aspirin cause gastric ulcers. I3C has been reported as antiulcer agent. However, DIM potential to mitigate the ulcer has to be evaluated.

6.3 Impact on the treatment of ischemic stroke

Both I3C and DIM exhibited antiplatelet and antithrombotic mechanisms in ameliorating ischemic stroke. Precisely, DIM shows potent amelioration of platelet aggregation and thrombus generation. Moreover, DIM exhibited the equipotent amelioration of ischemic stroke-induced pathological abnormalities when compared with CLOP. Therefore, I3C and DIM can be used as primary treatments as antiplatelet and antithrombotic agents as well as a neuroprotectant in the secondary management of ischemic stroke.