6.1 Summary and Conclusion

Stroke ranked, first to cause disability, second to cause dementia and third to cause death throughout the world. Stroke is of three types; ischemic, hemorrhagic and transient ischemic attack, out of which ischemic stroke alone account for more than 80 % of clinical cases. Treatment schedule of ischemic stroke follows long-term therapy of antiplatelet drugs such as aspirin and clopidogrel. However, long-term treatment with these antiplatelet drugs causes a severe adverse effect. Thus, the development of novel therapy is required for the management of cerebral ischemia. Piracetam is a nootropic antiplatelet drug which ameliorates ischemic injury by improving blood flow and oxygen availability in the brain. Piracetam can be used as an alternative of aspirin as it has less adverse effect compared to existing antiplatelet drugs. Reports suggested that treatment with piracetam prevents secondary stroke. However, there is no report on blood-brain penetration and CNS availability of piracetam during stroke condition. Thus, the pharmacokinetics and brain penetration of piracetam during ischemic condition would aid to improve pharmacotherapeutics in ischemic stroke. Ischemic stroke was induced by middle cerebral artery occlusion for 2 h in male Wistar rats followed by reperfusion. After 24 h of middle cerebral artery occlusion or 22 h of reperfusion, piracetam was administered for pharmacokinetic, brain penetration, and pharmacological experiments. In the pharmacokinetic study, blood samples were collected at different time points after 200-mg/kg (oral) and 75-mg/kg (intravenous) administration of piracetam through the right external jugular vein cannulation. In brain penetration study, the cerebrospinal fluid, systemic blood, portal blood, and brain samples were collected at pre-designated time points after 200-mg/kg oral administration of piracetam. In a separate experiment, the pharmacological effect of the

single oral dose of piracetam in middle cerebral artery occlusion was assessed at a dose of 200 mg/kg. All the pharmacokinetic parameters of piracetam including area under the curve (AUC_{0-24}), maximum plasma concentration (C_{max}), time to reach the maximum plasma concentration (t_{max}), elimination half-life ($t_{1/2}$), the volume of distribution (V_z), total body clearance, mean residence time, and bioavailability were found to be similar in ischemic stroke condition except for brain penetration. Piracetam exposure (AUC_{0-2}) in brain and CSF was found to be 2.4 and 3.1 fold higher, respectively, in ischemic stroke compared to control rats. Piracetam significantly reduced infarct volume by 35.77% caused by middle cerebral artery occlusion. Hence, brain penetration is not the limiting factor for the suboptimal efficacy of piracetam in the treatment of cerebral stroke. Further, the monotherapy of piracetam is not potent enough in the prevention of secondary stroke, and piracetam is less efficacious than aspirin in the treatment of cerebral stroke because it acts through multiple mechanisms. Thus we evaluated other potential compounds for the treatment of cerebral stroke.

A biomaterial is any substance that has been engineered to interact with biological systems for a medical purpose - either therapeutic (treat, augment, repair or replace a tissue function of the body) or diagnostic agent. Bioactive glass is a third-generation biomaterial (regenerate & repair biological tissue) clinically used in bone and dental repair. Professor Larry Hench developed bioactive glass at the University of Florida in the late 1960s. The first bioactive glass used by Hench was 45S5 which is named as bioglass™ having following composition in Mole % SiO_2 (46.1), Na_2O (26.9), CaO (24.4) and P_2O_5 (2.6). Bioactive glasses have benefited over current antiplatelet drugs as it is biocompatible and helpful in the treatment of the hemorrhagic condition, wound healing and duodenal or gastric ulcer, which are the main

adverse event of antiplatelet therapy. Bioactive glass is safe in humans as it has four FDA approvals, out of which three FDA approvals are for the dental and bone repair, and one is for liver cancer treatment. In liver cancer treatment, bioactive glass is used as a carrier for the delivery of radioactive substance yttrium-90. In the current study, we have used barium, and silver doped bioactive glasses as both silver and barium inhibits platelet aggregation and hold potential to be promoted as antiplatelet/antithrombotic agents. Silver inhibited ADP induced platelet aggregation by accumulating within platelet granules and reduces interplatelet proximity while barium blocks IRKs channels (Inwardly rectifying potassium channels) which regulate ADP-induced platelet aggregation. Further earlier studies reported that doping of metals like barium & silver in bioactive glasses significantly enhanced its VEGF stimulant property. Furthermore, people used to take a sand bath, natural therapy for improving blood circulation. Sand contains silica similar to bioactive glass. Therefore, we have hypothesized the application of bioactive material in the treatment of cerebral stroke. We found the antithrombotic activity and inhibitory effect of barium containing bioactive glass (BaBG) and silver-containing (AgBG) on platelet aggregation in both in-vitro as well as ex-vivo. Both above bioactive glasses were effective in ameliorating middle cerebral artery occlusion (MCAO) induced neurobehavioral impairment, restored the functional brain activity, % infarction, cerebrovascular hemodynamic, reduced the histopathological changes and increases vascular endothelium growth factor.

We have shown the pharmacology of both BaBG and AgBG in the treatment of cerebral stroke. As we found that it is pharmacologically active; therefore, in another study, we have performed the acute and subacute toxicity of BaBG and AgBG. We have done single-dose acute toxicity study to determine the LD_{50} dose of BaBG and AgBG. Further, to determine

the maximum safe dose level, we have performed a 28-days repeated-dose subacute toxicity study initiated with the highest therapeutic dose followed by 10 and 50 mg/kg in rats. The animal was observed for changes in physical appearance, food intake, water intake, body weight gain, cardiovascular parameters, the blood flow of highly perfused organs, organ weight coefficient, pathological and complete blood parameters. BaBG at 10 mg/kg dose and AgBG at 5 mg/kg were found to be safe if given intravenously for 28 days daily. Further, the LD_{50} of the BaBG was found to be more than 2000 mg/kg, and for AgBG it was less than 300 mg/kg suggesting that BaBG is safe compared to AgBG. Thus, the present study demonstrates a safe dose of BaBG and AgBG, which can be utilized in future.

Patients of cerebral stroke are more prone to have ischemic ulcer due to the disturbance in vascular homeostasis and continuation of antiplatelet therapy further worsens the condition. As it is well known that long term treatment with aspirin (antiplatelet) caused gastric bleeding as a serious adverse effect. Therefore, in the present study, we have also shown the protective application of BaBG against celiac artery occlusion (CAO) and other gastrointestinal ulcer models. Prophylactic effect of BaBG pretreatment was evaluated for 5 days in ischemic, ethanol, aspirin and pyloric ligation-induced gastric ulcer and cysteamine-induced duodenal ulcer models. Repeated treatment of 10 days of BaBG was evaluated in the healing ulcer model of acetic acid. BaBG significantly reduced the ulcerative damage against all the six tested ulcer models. Scanning electron microscope (SEM) images have shown that BaBG forms a physical protective barrier over the gastroduodenal epithelium cell. In the ischemic, pyloric-ligation, ethanol and aspirin models, BaBG showed significantly increased gastric pH, indicating antacid like activity. Thus, BaBG mediates antiulcer action by forming a protective physical barrier against harsh luminal factors and acid neutralization.

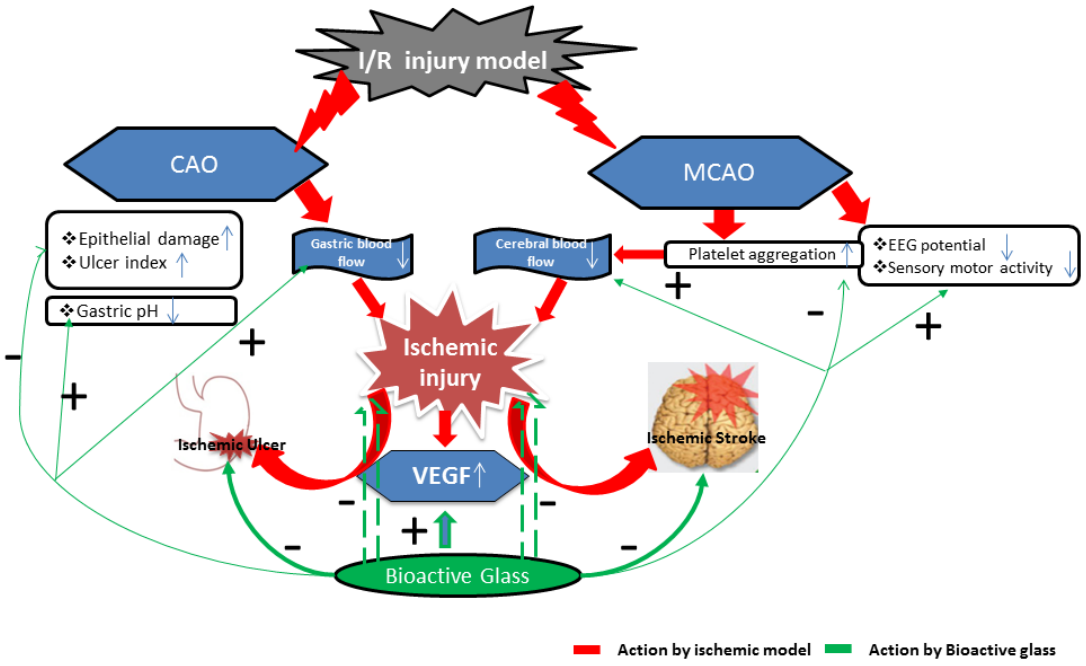

Figure 6.1 Proven hypothesis. "—" denotes inhibition and "+" denotes activation

In summary, the brain exposure of piracetam increases in pathological conditions of ischemic stroke, suggesting that brain penetration may not be the limiting factor for the activity of piracetam. Further, characterization and evaluation (safety & efficacy) of medicated bioactive glasses (BaBG/AgBG) show antiplatelet and antithrombotic activity and pharmacological role in the treatment of cerebral ischemia. Toxicity studies showed that BaBG is safe while AgBG is mild toxic at a very high dose. Furthermore, BaBG shows significant anti-ulcer activity in ischemic and other acute as well as chronic ulcer models which can be an advantage compared to most widely used antiplatelet drug aspirin.