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Glucagon-like peptide-1 receptor agonists as neuroprotective agents for ischemic stroke: a systematic scoping review

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Abstract

Stroke mortality and morbidity is expected to rise. Despite considerable recent advances within acute ischemic stroke treatment, scope remains for development of widely applicable neuroprotective agents. Glucagon-like peptide-1 receptor agonists (GLP-1RAs), originally licensed for the management of Type 2 Diabetes Mellitus, have demonstrated pre-clinical neuroprotective efficacy in a range of neurodegenerative conditions. This systematic scoping review reports the pre-clinical basis of GLP-1RAs as neuroprotective agents in acute ischemic stroke and their translation into clinical trials. We included 35 pre-clinical studies, 11 retrospective database studies, 7 cardiovascular outcome trials and 4 prospective clinical studies. Pre-clinical neuroprotection was demonstrated in normoglycemic models when administration was delayed by up to 24 h following stroke induction. Outcomes included reduced infarct volume, apoptosis, oxidative stress and inflammation alongside increased neurogenesis, angiogenesis and cerebral blood flow. Improved neurological function and a trend towards increased survival were also reported. Cardiovascular outcomes trials reported a significant reduction in stroke incidence with semaglutide and dulaglutide. Retrospective database studies show a trend towards neuroprotection. Prospective interventional clinical trials are on-going, but initial indicators of safety and tolerability are favourable. Ultimately, we propose that repurposing GLP-1RAs is potentially advantageous but appropriately designed trials are needed to determine clinical efficacy and cost-effectiveness.

Keywords

Acute stroke, neuroprotection, reperfusion, clinical trials, animal models

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Introduction

Stroke accounts for 6.5 million deaths per year globally and by 2030 will result in an annual loss of over 200 million disability-adjusted life years.[1,2] With an increasing number of strokes occurring in younger patients, alongside an increased number of stroke survivors, the cost of post-stroke care is rising. There is, therefore, significant scope to improve upon the current position.

Considerable advances have been made in acute ischemic stroke (AIS) treatment, notably reperfusion therapies, but these are limited to 10–20% of total stroke patients following careful clinical and radiological selection.[3] Even when intravenous thrombolysis and/or endovascular thrombectomy are administered,

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reduction in disability is highly time dependent.[4,5] Scope remains for further improvement, especially for patients who are unsuitable for reperfusion therapies or those within remote environments.

Using simpler clinical selection processes, neuroprotective therapies could bring benefits to a wider patient group. Neuroprotectants could also enhance the benefits of reperfusion therapies by preservation of the ischemic penumbra and reduction in ischemic reperfusion injury. Despite many demonstrating pre-clinical potential, a suitable agent has not yet been identified by translational studies.[6] There remains a multitude of factors affecting the translation from bench-to-bedside. Namely, animal models are not perfect in their representation of the heterogeneity of clinical stroke.[7] Stroke in humans occurs in the context of ageing, co-morbidity (hypertension, diabetes mellitus, atrial fibrillation, pre-existing cerebrovascular disease) and concomitant medication use.[8] Furthermore, factors such as gender, cerebral blood flow, body temperature and glycemic status may influence stroke mechanism and outcomes associated with therapy.[6,9–11]

Glucagon-Like Peptide-1 (GLP-1) receptor agonists are gaining increasing momentum as possible neuroprotective agents in AIS. GLP-1 is an incretin hormone. Alongside its role in insulin secretion from the pancreas and glucagon suppression, it also crosses the blood-brain barrier (BBB) and promotes synaptic function, enhances neurogenesis, reduces apoptosis and protects neurons from oxidative stress.[12] GLP-1 is produced in the brain and receptors are distributed throughout the central nervous system.[13] GLP-1 Receptor Agonists (GLP-1RAs), licensed for Type 2 Diabetes Mellitus (T2DM) have already demonstrated pre-clinical neuroprotective efficacy in Alzheimer's Disease and clinical trials in neurodegenerative conditions are ongoing.[12,14]

The aim of this systematic scoping review is to report the pre-clinical basis of GLP-1RAs as neuroprotective agents in AIS and their translation into clinical trials. In addition to describing the characteristics and quality of studies, the objectives are to specifically consider timing of administration, association with glycemic status, neuroprotective outcomes and application to clinical care.

Materials and methods

Eligibility criteria

Pre-clinical: We included pre-clinical *in vivo* studies which administered naturally occurring GLP-1, a mimetic or analogue, before, during or after stroke induction. Normoglycemic, hyperglycemic and induced T2DM models were included.

Studies were excluded if their only focus was hemorrhagic stroke as this does not reflect the proposed mechanism for how GLP-1 is involved in ischemic tissue injury. Those studies which reported incidence of hemorrhagic transformation as a complication of AIS were included as these reflect post-stroke complications.

Clinical: We included all prospective clinical trials which administered GLP-1RAs before, during or after stroke onset with outcome measures defined to identify neuroprotective efficacy by way of stroke volume reduction or improvement in post-stroke function or mortality. We also included any potential feasibility or safety-based studies in this area.

Our scoping searches identified that very few prospective clinical trials measuring stroke outcomes were available. Pragmatically, we therefore also included all retrospective database analyses of stroke incidence or composite cardiovascular outcomes in patients treated with GLP-1RAs. Furthermore, we included cardiovascular outcome trials (CVOTs) of GLP-1RAs to evaluate the incidence of stroke in this relatively higher risk cohort.

Studies were excluded if their full-text was not available or not published in English. Efforts were made to contact authors directly to obtain any missing articles or data.

Database search strategy

After several initial scoping searches,[15] we accessed Web of Science on 19 March 2020 to search MEDLINE, Web of Science core collection, BIOSIS and SciELO from 1 January 2000. Keywords were EITHER 'GLP(-)1, glucagon like peptide(-)1, exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, semaglutide' AND EITHER 'stroke, CVA, cerebrovascular, h(a)emorrhage, small vessel disease'. Articles were cross-referenced and references were searched to identify further studies of interest.

All articles/studies were screened independently in an unblinded, standardised manner by MM and HE by way of title and abstract to identify those suitable for full-text review. Queries and disagreements were resolved by discussion. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were applied. Pre-clinical studies were appraised according to Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines,[16] and the updated Stroke Therapy Academic Industry Roundtable Preclinical Recommendations (STAIR) guidelines.[7] Data supporting the findings of this review are available from the corresponding author upon reasonable request.

Results

Study selection

The literature search identified 797 results (see Figure 1) alongside 10 from other sources. After removal of duplicates, this left 794 for screening. We excluded 593 articles based upon title and review of abstract leaving 201 full text articles to review. In total, 35 preclinical studies, 11 retrospective database studies, 7 cardiovascular outcome trials and 4 prospective clinical studies met the inclusion criteria.

Pre-clinical studies

Characteristics of included studies. As shown in Table 1, 35 pre-clinical studies were included within this review. Studies were completed between 2009 and 2020. Studies were predominantly based upon mouse and rat models of stroke; however, one study utilised a gerbil model.[17] Stroke induction was either via transient (range 30–120 min) or permanent common carotid (CCAO) or middle cerebral artery occlusion (MCAO). Most studies induced unilateral occlusion in keeping with spontaneously occurring stroke onset in humans, but six studies utilised a bilateral occlusion model. Cerebral ischemia was induced by either ligation, filament occlusion or ablation of the relevant artery.

Twelve studies administered exendin-4,[17–28] nine used liraglutide,[29–37] three used rhGLP-1 (recombinant human GLP-1),[38–40] three used lixisenatide[41–43] and one study each reported the utility of semaglutide,[43] PEx-4 (exendin-4 loaded poly-microspheres),[44] proGLP-1 (long acting GLP-1RA),[45] DMB (GLP-1R agonist/modulator),[46] dual GLP-1/Glucose-dependent Insulinotropic Peptide (GIP) agonist (GLP-1/GIP DA),[47] oxyntomodulin (co-activates GLP-1R and glucagon receptor),[48] P7C3 (aminopropyl carbazole compound)[49] and one study directly compared exendin-4 with liraglutide.[50] In eight studies, GLP-1R antagonists, such as Ex-9-39, were administered to study the role of the GLP-1R in neuroprotective mechanisms.[19,21–23,41,45,46,49]

Two studies investigated multiple doses of GLP-1RAs to compare neuroprotective efficacy and concluded that neuroprotection was dose-dependent.[20,51]

Most studies administered GLP-1RAs via intraperitoneal, subcutaneous or transvenous routes. However, Zhang et al. reported neuroprotection with both oral DMB[46] and intranasal exendin-4.[22]

Some 14 studies administered GLP-1RAs chronically prior to the onset of stroke. Clinically, this would represent those patients who receive GLP-1RAs as part of routine T2DM management and then go on to experience AIS.[18,19,22,23,29,31,38,39,42,44–46,48,51] Chronic pre-treatment occurred

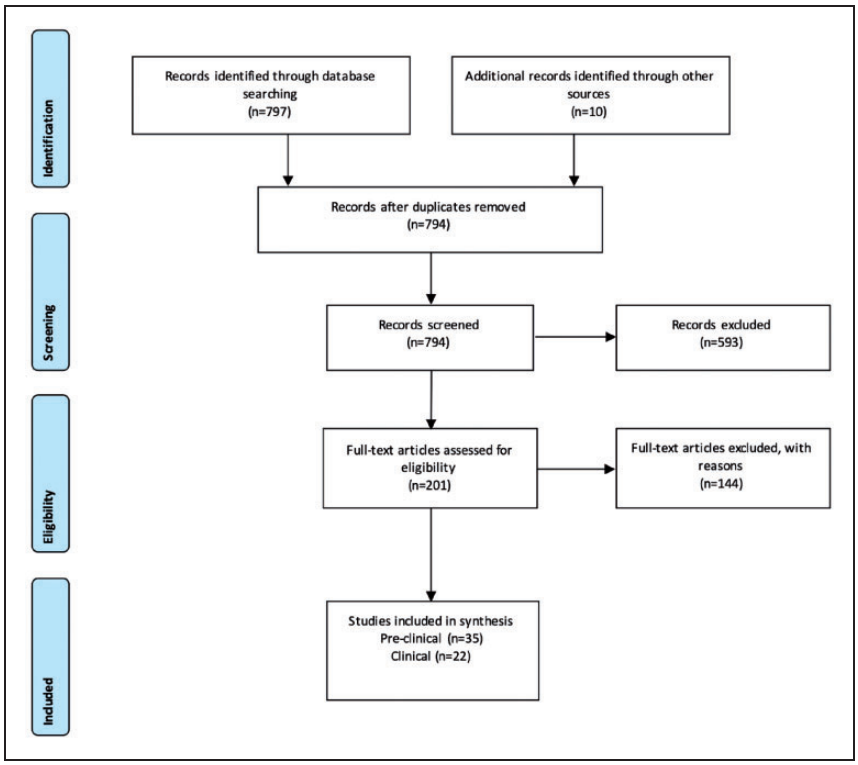

Figure 1. PRISMA flow chart demonstrating the selection of studies.

Table 1. Overview of the pre-clinical studies.

Author	Year	Animal model	Co-morb.	Drug	Admin route	Sub-studies	Stroke induction (time/min)	Administration time (min)		Main outcomes (NP – Neuroprotective)
								Pre-ischemia	*Post-ischemia*	
GLP-1RA administered prior to stroke onset (including chronic pre-treatment)										
Li et al.[18]	2009	Rat		Ex-4	i.c.v.		u.l tMCAO (60)	15		NP – ↓ infarct volume and improves functional outcome
Briyal et al.[19]	2012	Rat		Ex-4	i.p.	BQ123	u.l. pMCAO	BD – 7 days		NP – ↓ infarct volume, ↓ motor deficit, ↓ oxidative stress parameters (↓ MDA, ↑ GSH, ↑ SOD) NP via ↓ oxidative stress and independent of endothelin receptor.
Briyal et al.[29]	2014	Rat	DM	Liraglutide	s.c.	Non-diabetic, insulin	u.l. pMCAO	OD – 14 days		NP – ↓ infarct volume, ↓ motor deficit, ↓ oxidative stress parameters (↓ MDA, ↑ GSH, ↑ SOD), ↓ apoptosis (↑ Bcl-2, ↓ Bax). NP in both non-diabetic & DM models with similar infarct volume reduction, insulin treatment did not reduce infarct volume.
Chien et al.[44]	2015	Rat	DM	PEx-4	s.c.	Non-diabetic, Ex-4	b.l tCCAO (10) & hypotension	OD – 14 days		NP – ↓ brain oedema, ↓ cognitive deficit, ↓ oxidative stress, ↑ cerebral blood flow. PEx-4 more NP than Ex-4 in DM.
Zhang et al.[45]	2015	Mouse		Pro-GLP-1	i.p.	shRNA	u.l. tMCAO (90)	OD – 7 days		NP – ↓infarct volume, ↓ neurological deficit, ↓ apoptosis (↑ Bcl-2, ↓ Bax). NP not glucose dependent. NP blocked by shRNA (suggesting GLP-1R mediated NP)
Jiang et al.[38]	2016	Rat	DM	rhGLP-1	i.p.	nimodipine, insulin	u.l. tMCAO (90)	TDS – 14 days		NP in DM– ↓ infarct volume (more than insulin group), ↓ neurological deficit, ↓ brain injury markers (↓ S100B, ↓ NSE, ↓ MBP)
Zhang et al.[46]	2016	Mouse		DMB	p.o.	Ex-4, Ex-9-39, shRNA	u.l. tMCAO (60)	30		NP given orally– ↓ infarct volume, ↓ neurological deficit, ↓ apoptosis (↑ Bcl-2, ↓ Bax). No impact on plasma insulin and glucose levels in non-diabetic mice. DMB activation of GLP-1R different to Ex-4. NP blocked by shRNA but not by Ex-9-39.
Zhang et al.[22]	2016	Mouse		Ex-4	i.n.	Intraperitoneal Ex-4, shRNA	u.l. tMCAO (90)	OD – 7 days		NP given intranasally – ↓ infarct volume, ↓ neurological deficit, ↓ apoptosis (↓ caspase-3) Intranasal route produced ↑ CNS concentration than intraperitoneal No impact on plasma insulin and glucose levels and NP was blocked by shRNA suggesting NP is GLP-1R mediated.
Kim et al.[23]	2017	Rat		Ex-4	i.c.v	Ex-9-39	u.l. MCAO (60)	30		NP – ↓ infarct volume (by up to 75%), ↓ neurological deficit, ↓ oxidative damage/ inflammation (↓ COX-2, ↓ PE2)
Li et al.[48]	2017	Rat		OXM	i.c.v.		u.l. tMCAO (60)	15		NP – ↓ infarct volume, ↑ locomotive activity
Abdel-latif et al.[42]	2018	Rat	DM	Lixisenatide	i.p.	glimepiride	b.l. tCCAO (30)	OD – 14 days		NP – ↓ infarct volume, ↓ neurological deficit, ↓ oxidative stress (↓ MDA, ↑ GSH, ↑ catalase), ↓ inflammation/apoptosis (↓ caspase-3, ↓ TNF-α). Lixisenatide more NP than glimepiride.
Fang et al.[39]	2018	Rat	DM	rhGLP-1	i.p.	nimodipine	u.l. tMCAO (90)	TDS – 14 days		NP – ↓ infarct volume, ↓ neurological deficit, ↓ oxidative stress parameters (↓ MDA,↑ GSH, ↑ SOD), ↓ apoptosis (↑ Bcl-2, ↓ Bax, ↓ caspase-3), ↑EAAT2,

(continued)

Table 1. Continued.

Author	Year	Animal model	Co-morb.	Drug	Admin route	Sub-studies	Stroke induction (time/min)	Administration time (min)		Main outcomes (NP – Neuroprotective)
								Pre-ischemia	*Post-ischemia*	
Filchenko et al.[31]	2018	Rat	DM	Liraglutide	s.c.	Non-diabetic, metformin	u.l. tMCAO (30)	OD – 7 days		NP – ↓ infarct volume, ↓ neurological deficit only in non-DM model, NP not associated with glycemic control amelioration (Metformin not NP)
Gad et al.[51]	2020	Rat		Lixisenatide	i.p.	0.7 and 7 nmol/kg lixisenatide	b.l. tCCAO (60)	OD – 14 days		NP – ↓ oxidative stress parameters (↓ MDA, ↑ GSH, ↑ SOD), ↓ apoptosis (↑ Bcl-2, ↓ Bax, ↓ caspase-3), ↓ inflammatory markers (↓ MPO, ↓ IL-1β, ↓ TNF-α), ↑ viable hippocampal neurons on histological staining. Higher dose Lixisenatide ↑ NP
GLP-1RA administered prior to and following stroke onset										
Hyun Lee et al.[17]	2011	Gerbil		Ex-4	i.p.		b.l. tCCAO (5)	120	60	NP – ↓ neurological deficit, ↑ GLP-1R immunoreactivity, ↓ microglial activation
Darsalia et al.[20]	2012	Rat	DM	Ex-4	i.p.	0.1, 2 or 5μg/kg of Ex-4	u.l. tMCAO (90)	BD – 4 weeks	BD –2–4 weeks	NP – ↓ infarct volume (no difference between 2 & 4 weeks post MCAO), ↓ microglial infiltration but marginal effect on activation, ↑ stem cell proliferation & neuroblast formation. NP is dose dependent.
Jia et al.[21]	2015	Rat		Ex-4/Catapol	i.c.v.	Ex-9-39	u.l. tMCAO (60)	15	0	NP – ↓ infarct volume, ↓ neurological deficit, ↑ hippocampal β-endorphin expression, NP blocked by Ex-9-39, β-endorphin anti-serum and naloxone
Deng et al.[30]	2018	Rat	DM	Liraglutide	i.p.	Non-DM	u.l. pMCAO	BD – 7 days	BD – 7 days	NP- ↓ infarct volume, ↓ neurological deficit, ↓ oxidative stress (↑ SOD), ↓ inflammation (↓ MPO), ↑ Nrf2, ↑ HO-1 (antioxidative stress signalling pathway) No significant difference in NP between DM & non-DM
GLP-1RA administered following stroke onset (including delayed administration)										
Teramoto et al.[24]	2011	Mouse		Ex-4	t.v.		u.l tMCAO (60)		0,60,180	NP – ↓ infarct volume, ↓ neurological deficit, ↓ oxidative stress, ↓ inflammation (↓ microglial activation), ↑ intracellular cAMP levels (due to GLP-1R activation). NP probably mediated via raised intracellular cAMP levels
Sato et al.[32]	2013	Rat		Liraglutide	i.p.		u.l. tMCAO (90)		60	NP – ↓ infarct volume, ↓ neurological deficit, ↓ oxidative stress, ↑ VEGF in cortex but not in the striatum.
Darsalia et al.[25]	2014	Mouse	DM	Ex-4	i.p.	2 month old & 14 month obese/DM mice	u.l. tMCAO (30)		90, 180 or 270 then OD 1 week	NP – ↑ neuronal survival, ↑ M2 microglial markers Non statistically significant reduction in pro-inflammatory markers. NP in non-DM/DM at 50μg/kg at 90/180 min and at 90 min for 5μg/kg. Stroke volume not affected by Ex-4 administration. Not NP when Ex-4 administered 4.5hrs after stroke onset. NP in both young and old animal models
Zhao et al.[40]	2015	Rat	DM	rhGLP-1	i.p.	nimodipine	u.l. tMCAO (120)		0	

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