Novel calcium-regulating mechanism of barium-doped material for the treatment of neuropathic pain

Thesis submitted in partial fulfilment for the Award of Degree

Doctor of Philosophy

By

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ACC	:	Anterior cingulate cortex
ACN	:	Acetonitrile
ALP	:	Alkaline phosphatase
ALT	:	Alanine transaminase
AMY	:	Amygdala
AST	:	Aspartate transaminase
ASTM	:	American society for testing and materials
AUC	:	Area under curve
$Ba(NO_{3)2}$	:	Barium nitrate
BaBG	:	Barium-doped bioactive glass
BBB	:	Basso, Beattie, and Bresnahan
BCA	:	Bicinchoninic acid
BET	:	Brunauer–Emmett–Teller
BJH	:	Barrett-Joyner-Halendra
$Ca(NO_3)_2.4H_2O$	:	Calcium nitrate tetrahydrate
CCBs	:	Calcium channel blockers
CCI	:	Chronic constriction injury
CCSEA	:	Committee for the Control and Supervision of Experimentals on Animals
CL	:	Clearence
C_{max}	:	Maximum plasma concentration
CMC	:	Carboxymethyl cellulose
CNS	:	Central nervous system
CRE	:	Creatinine
D	:	Day
EDTA	:	Ethylene diamine tetra acetic acid
EDX	:	Energy dispersive Xray
ETS	:	Experimental toe spreading
FBS	:	Fetal bovine serum
FDA	:	Food and Drug Administration
FTIR	:	Fourier transform infrared spectroscopy
GFAP	:	Glial fibrillary acidic protein
GIT	:	Gastro intestinal tract
HA	:	Hydroxyapatite
Hb	:	Haemoglobin
HCA	:	Hydroxy carbonated appetite
HCT	:	Haematocrit
Iba-1	:	Ionized calcium-binding adapter molecule 1
ICG	:	International Commission on glass
ICP-MS	:	Inductively coupled plasma mass spectrometry

IL 10	:	Interleukin 10
IL-6	:	Interleukin 6
IR	:	Infrared
IUPAC	:	International Union of pure and applied Chemistry
KBr	:	Potassium Bromide
kV	:	Kilo volt
LD_{50}	:	Lethal dose
LDH	:	Lactate dehydrogenase
LPS	:	Lipopolysaccharide
LTP	:	Long term potentiation
mA	:	Milli ampere
MBP	:	Myelin basic protein
MCHC	:	Mean cell haemoglobin concentration
MCV	:	Mean corpuscle volume
MHC	:	Major histocompatibility complex
MRT	:	Mean Residence Time
$NaNO_3$	:	Sodium nitrate
NBO	:	Non bridging oxygen
NF	:	Neurofilament
NF-κB	:	Nuclear factor kappa B
NOAEL	:	No observed adverse effect level
NP	:	Neuropathic pain
NPL	:	Normal foot print length
NTS	:	Normal toe spreading
OECD	:	Organization for Economic Cooperation and Development
OFT	:	Open field test
PBS	:	Phosphate buffer saline
PL	:	Print length
PNS	:	Peripheral nervous system
PPM	:	Parts per million
qRT-PCR	:	Quantitative Real-Time polymerase chain reaction
RAGE	:	Receptor for advanced glycated end product
RBC	:	Red blood corpuscle
RDW	:	Red blood cell distribution
RVM	:	Rostroventral medulla
SAED	:	Selected area electron diffraction
SBF	:	Simulated body fluid
SC	:	Spinal cord
SD	:	Standard deviation
SEM	:	Scanning electron microscopy
SFI	:	Sciatic functional index
SN	:	Sciatic nerve

$t_{1/2}$	:	Half life
TBI	:	Traumatic brain injury
TCO4	:	Technical committee 4
TEM	:	Transmission electron microscopy
TEOS	:	Tetraethyl orthosilicate
TEP	:	Triethyl phosphate
TH	:	Thalamus
TLR 4	:	Toll like receptor 4
T_{max}	:	Total time required to reach maximum plasma concentration
TMZ	:	Temozolomide
TNF-α	:	Tumour necrosis factor-alpha
TRIS	:	Trihydroxy methyl aminomethane
TRPV1	:	Transient receptor potential Vanilloid-1
TS	:	Total spreading
TT	:	Distance between intermediary toes
VEGF	:	Vascular endothelial growth factor
VGCC	:	voltage-gated calcium channel
V_z	:	Volume of distribution
WBC	:	White blood cells
XRD	:	X-ray diffractometry

PREFACE

The thesis's research work entitled "**Novel calcium-regulating mechanism of barium-doped material for the treatment of neuropathic pain**" is based on the assessment of the calcium-regulating mechanism of barium-doped biomaterial and its pharmacological potential for the treatment of neuropathic pain (NP) using chronic constriction injury (CCI) model. NP is a prominent clinical illness that arises as a consequence of injury to the somatosensory nervous system leading to either spontaneous or evoked pain. Globally, NP affects 7-10 % of people worldwide. Despite over a century of studies on nociception, the current treatments for chronic pain have limited effectiveness with adverse effects, leading to withdrawal and poor quality of life. Currently, calcium channel blockers (CCBs) are the first-line treatment for NP. The clinically prescribed CCBs include pregabalin and gabapentin which are organic in nature. Unlike inorganic compounds, organic compounds have various protein interactions which are linked to significant adverse effects. Therefore, it is imperative to develop novel therapeutic strategies that would work on calcium channels. We have selected an inorganic compound i.e., barium-doped bioactive glass (BaBG) that may have certain advantages over the organic compounds as barium that is doped in BaBG are endogenously present in the body so they may not pose any undesired effects. Besides, barium is present in exoplanets so we have evolved containing barium within us. Thus, BaBG was synthesized using the wet chemistry method i.e., sol-gel method. By using the *ex vivo* electrophysiological technique, the calcium-regulating properties of BaBG via its action on the calcium channel were assessed. Further, to examine its potential regenerative and anti-inflammatory properties, cell line-based studies were performed. To assess the therapeutic potential of BaBG for the treatment of NP, a

comprehensive calcium-modulating molecular mechanism was performed in CCI-induced NP in rats. In extension, the sensory and motor abnormalities developed post-CCI was evaluated using a variety of pain behavioral assessment tests. The synaptic rewiring occurring post-BaBG treatment was also examined by tracing the neurons. Furthermore, the *in vivo* biodistribution of the dopants leached from BaBG was determined using quantitative analytical techniques such as inductively coupled plasma mass spectrometry (ICP-MS) as BaBG not only contains barium under the permissible physiological limits but also has other elements that are involved in various physiological activities. By employing a multidisciplinary approach encompassing materials science, pharmacology, and toxicology, we strived to harness the unique properties of BaBG for the development of therapeutic interventions for NP. The entire work has been compiled into seven chapters**: Chapter 1** describes the introduction and significance of the present study. **Chapter 2** describes the synthesis and characterization of barium-doped bioactive glass (BaBG), investigating it's *in vitro* regenerative and anti-inflammatory potential. **Chapter 3** describes the *in vivo* pharmacokinetics, biodistribution, and excretion of dopants released from BaBG following oral administration. **Chapter 4** describes the oral acute and sub-acute toxicity study of BaBG according to the OECD guidelines. **Chapter 5** describes the temporal changes in the intracellular calcium and S100b protein levels in the pathophysiology of CCI-induced NP in rats. **Chapter 6** describes the pharmacological evaluation of BaBG in the treatment of NP. **Chapter 7** summarizes the entire study completed with its essential outcomes.