APPENDIX S1

Background to the Calmodulin (CaM)/nitric oxide (NO)/cyclic guanosine monophosphate signalling pathway

NO is a soluble and extremely reactive gas that is produced by certain cells, diffuses through cell membranes, triggering various local processes in other cells. NO is continuously synthesized from the amino acid L-arginine in endothelial cells by the constitutive calcium (Ca^{2+}) and CaM-dependent enzyme (known as NO synthase (NOS)) **(1)**. The fact that biochemical metabolic pathways are apparently responsible for the effect of electromagnetic impulses has been investigated and proven for several years **(2-5)**. A simple electrical model for living cells predicts an increasing probability for electric field interactions with intracellular substructures of both prokaryotic and eukaryotic cells when the electric pulse duration is reduced into the sub-microsecond range **(6)**. Electromagnetic fields act as a first messenger substance in biological signal transmission, with an influence on CaM-dependent signal transmission for tissue regeneration **(7-9)**. CaM which is a Ca^{2+}-binding regulatory protein that is highly conserved in all eukaryotes, plays an important role as a second messenger responsible for activating proteins **(10)**.

Local vascular tone and blood pressure are controlled by several auto regulatory functions of blood vessels **(11)**. Central among them is the ability of the endothelium to local sense fluid shear stress exerted by the flowing blood and to respond to it with the release of vasodilatory factors such as NO **(12)**. Flow-induced vasorelaxation mediated by NO is important for the local adaptation of vessel diameter to blood flow, and also controls vascular tone and blood pressure **(13)**. The activity of NOS is increased by fluid shear stress through various, mechanisms (***eg***; vibration, heat or electromagnetic waves) **(14, 15)**. Whereas an acute increase in flow results in a transient elevation of intra-cellular Ca^{2+} and the subsequent Ca^{2+}/CaM-dependent activation of endothelial NOS (eNOS) **(15-17)**, a sustained flow-induced NO formation requires phosphorylation of the enzyme **(14-16)**. eNO is excreted for vascular relaxation, to increase blood flow and to downregulate the inflammatory cascade, including those that occur in pain **(18-20)**. As previously described one previous study reported that patients with back pain have three-fold higher level of NO in the perifacial region compared to the healthy controls **(21)**. CaM-dependent NO signalling is involved in the cell and tissue response to weak non-thermal electromagnetic field signals **(22)**. Non-thermal pulsed electromagnetic fields, from low frequency to pulse-modulated radio frequency, have been successfully employed as adjunctive therapy for the treatment of delayed and non-union fractures, fresh fractures, chronic wounds, and acute and chronic pain events **(22, 23)**.

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