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Efficacy of Sustained-Release Novel Bupivacaine Formulations in Sheep

A thesis presented in partial fulfilment of the requirements for
the degree of

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Abstract:

The objective of this thesis was to prepare and assess several formulations of the local anaesthetic bupivacaine to achieve a longer duration of action. Intralipid® emulsion (a soybean oil emulsion) and collagen combined with titanium oxide nanoparticles were used to develop slow release bupivacaine formulation. These formulations were tested both *in vitro* as a pilot study and *in vivo* in sheep.

Collagen was extracted from bovine limed split hide (a by-product of the leather industry). The collagen as a 1% solution was mixed with bupivacaine hydrochloride 0.5% aqueous solution (Marcain® 0.5%, AstraZeneca, New Zealand) giving a final concentration of 0.25% bupivacaine. Intralipid® (20%, Fresenius Kabi Australia) and bupivacaine 0.5% were mixed resulting in a 0.25% bupivacaine lipid emulsion. Both formulations were tested in vitro pilot study for the release of bupivacaine through a dialysis membrane. The concentration of bupivacaine in the dialysate was measured using High-Performance Liquid Chromatography (HPLC). In the animal studies, 18 sheep were used to compare bupivacaine (control) and bupivacaine-Intralipid®, and another 18 sheep for commercial bupivacaine (control) and collagen- bupivacaine. Each sheep received a nerve block using the control or test formulation in each forelimb. The nerve block was placed at the level of the accessory digits with three injections totalling 4 mL using a 22G needle. The efficacy was tested by manually applying a mechanical noxious stimulus with a blunt instrument below the level of the block. This test was performed first after 15 min and then at one-hour intervals. The time at which a response was observed was considered as the end-point for that formulation.

In the *in vitro* pilot study, both collagen and Intralipid®-based formulations showed slightly more sustained release compared to the control group. However, collagen-based formulation of bupivacaine had the most sustained-release among all.

In the sheep study, the Intralipid®-based formulation significantly extended the duration of the nerve block compared to the control group ($P<0.05$). On the contrary, the collagen-based

formulation of bupivacaine shortened the duration of action significantly compared to control group ($P<0.05$).

In conclusion, an Intralipid®-based formulation provided a more sustained action after nerve blocks in the sheep metacarpal region compared to aqueous bupivacaine or the collagen based formulation. Further research on structure and activity of collagen and its interactions with bupivacaine is required to develop a longer acting formulation.

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Table 7.1 - Inter-Day Variations in Standard Group

Table 7.2 – Intraday Variations in Standard Group

Abbreviations:

Col	Collagen
HCL	Hydrochloric acid
HPLC	High-performance liquid chromatography
IVRT	<i>In vitro</i> drug release test
LLQ	lower limit of quantification
MNT	Mechanical nociceptive testing
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
NH ₄ Cl	Ammonium chloride
NP	Nanoparticle
PAA	Poly (acrylic acid)
PVP	Poly (vinylpyrrolidone)
UV	Ultra Violet
TiO ₂	Titanium oxide
ZnO	Zinc oxide