EFFECT OF LIDOCAINE AND SUBSTANCE P ON MIGRATION AND PROLIFERATION "IN VITRO" OF HUMAN ADIPOSE DERIVED STEM CELLS (hASCs) AND BREAST CANCER CELLS (MDA-MB-231)

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INTRODUCTION:

Local anaesthetics inhibit proliferation and migration while Substance P (SP) has demonstrated the opposite effect (1,2,3). Local anaesthetics seem to inhibit both SP binding and the related increase in intracellular calcium (4). Our objectives were:

To demonstrate migration and proliferation inhibition induced by lidocaine of hASCs and MDA-MB-231 breast cancer cells. And to demonstrate SP can reduce the effect of lidocaine on migration and proliferation of hASCs and MDA-MB-231 breast cancer cells.

METHODS:

Cells were treated with different doses of lidocaine (4000, 10000 y 20000 µM) and/or SP (50 y 500 nM). Migration was evaluated with a wound healing scratch assay after incubation at 37°C and 5% CO₂ for 24 hours. The wounded area was imaged at the beginning and at 12 hours and analyzed with Image J Software. The results are shown as a percentage of remaining denuded area.

Proliferation was evaluated with AlamarBlue reagent viability assay. After 24 hours, cells were treated with different doses of lidocaine (4000,10000 y 2000 µM) and/or SP (50 y 500 nM).

All assays were repeated (x3) and the percentage difference between treated and control cells were calculated. Kruskal-Wallis test was used to compare mean values and p<0,05 considered significant.

Table 1. Proliferation study

hASCs			MDA-MB-231	
Control %	Lido 20000	Lido 20000 + SP 50	Lido 20000	Lido 20000 + SP 50
100	30	66	30	62
100	32	65	30	61
100	31	64	28	61
100	31	65 *	29	61*

LIDOCAINE (uM) + SP (nM) %VIABILITY 24H. All results are media of 3 assays (x3). Last line presents the average of all studies ± SD. (*p<0.05)

RESULTS:

Lidocaine inhibited significantly migration at 4000µM compared with control of hASCs cells (p=0,05) and also of MDA-MB-231 (p=0,0001). Substance P did not influence migration alone or with lidocaine (Figure 1).

Lidocaine inhibited proliferation at 20000 µM compared with control of hASCs cells (p=0,0001) and also of MDA-MB-231 (p=0,0002). Adding substance P (50 nM) reduced the inhibition effect of lidocaine on viability of hASCs cells (p=0,03) and also of MDA-MB-231 (p=0,006) (Table1).

CONCLUSIONS:

Lidocaine inhibited migration and proliferation. Substance P partially antagonizes the inhibition of proliferation induced by lidocaine, but does not alter the decrease on migration.

References:

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Fig.1 Migration Assay. Migration was evaluated with a wound healing scratch assay. A cell monolayer was scratched with a 100 µl tip to form a wound and obtain a cell-free zone where cells at the edge can migrate and recover the wound in both cell line, hASCs (A1) and MDA-MB-231 (A2). The results are shown as a percentage of remaining denuded area. The * shows the significance relative to the control group, hASCs (B1) and MDA-MB-231 (B2) (p value of *<0.05; **<0.001 and ***<0.0001)