

Efficiency of transfection and intracellular localization of the FITC-labelled siRNA GL2 in human CSMCs. Just after the end of transfection, the intracellular localization (A) of the FITC-labelled siRNA GL2 transfected by either Cellfectin or Metafectene was documented in living cells to avoid artifacts introduced by fixation; under optimized conditions (2 hours of transfection, siRNA/transfecttant weight ratio of 1:2,5 and 1:1 for Cellfectin and Metafectene, respectively) a similar intracellular localization characterized by an even fluorescence in the cytoplasm and a positive fluorescence in the nucleus was observed; B) the amount of FITC positive cells was then evaluated by flow cytometry at different weight ratios siRNA- transfectant using an siRNA concentration of 230 nM and a transfection time of two hours; both transfectants displayed similar transfection efficacies. Data are expressed as mean \pm SEM, n = 4

Supplementary material -2



Comparison between the effects on target mRNAs by the different pairs of siRNAs.

Target mRNA levels were evaluated three days after siRNA delivery by Cellfectin (CSMCs from donor 1); 28S transcript levels served as normalizator; data are expressed as mean \pm SEM, n = 4. * p< 0.05 compared to control.

Supplementary material – 3



siRNA effects on CSMC amount

Three days after transfection, each specific siRNA (230 nM) delivered by Cellfectin was able to reduce the amount of CSMCs from donor 1 (A): cells were analysed by phase contrast microscopy, representative fields are shown; cumulative data from both donors are also reported (B), NTC = non treated cells, siGL2 = control siRNA treated cells; data are expressed as mean \pm SEM, n = 4. * p< 0.05 compared to control

<u>Supplementary material – 4</u>



siRNA effects on OAS1 mRNA levels and cell death

A) Three days after trasfection, none of the tested siRNAs (230nM) induced a relevant expression of interferon response-associated gene 2'-5'-oligoadenylate synthetase 1 (OAS1) in CSMCs (cumulative data from both donors, 28S transcript levels served as normalizator), NTC = non treated cells, siGL2 = control siRNA treated cells; data are expressed as mean \pm SEM, n = 6; the selected siRNAs neither induced any significant cytotoxicity as evaluated by lactate dehydrogenase (LDH; treatment by triton X-100, 1% final concentration, was introduced as positive control) test (B) nor apoptosis as evaluated by annexin V test (p> 0.1) (C); NTC = non treated cells; cumulative data from both donors are expressed as mean \pm SEM, n = 6