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IN VIVO EFFECTS OF PARTIAL PHOSPHOROTHIOATED ANTISENSE OLIGOS TARGETED AGAINST AT_1 RECEPTOR mRNA IN SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS

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Synthetic antisense oligodeoxynucleotides (ODNs) have been used successfully to inhibit many cell functions both *in vivo* and *in vitro* and offer the possibility to develop a new class of specific therapeutic agents. In order to prevent exonucleases hydrolisis of synthetic oligonucleotides a number of modifications of the phosphate backbone have been developed. Among others, phosphorothioate (PS) ODNs, where one of the oxigens in the phosphate backbone is replaced by a sulfur atom, have received increasing attention as reagents because of their improved resistence to enzymatic degradation. The renin-angiotensin system, involved in the homeostasis of blood pressure and cardiovascular functions through angiotensin II action on specific receptor subtypes, is a good model system where antisense molecules could be successfully tested. The AT_1 subtype receptor has been found to be implicated in most of the peripheral and cerebral actions of angiotensin II and results to be very abundant in hypothalamus and brainstem of spontaneously hypertensive rats (SHRs). In order to further investigate the effect of antisense molecules on the AT_1 level to control blood pressure we synthesized a 15 mer molecule, with the same nucleotide sequence of the PS molecule utilized by Gyurko (1), but containing only 3 phosphorothioate linkages at the 3' end of the molecule. This modification should protect the ODN by 3' esonucleases degradation while the remaning 11 phosphodiester internucleoside linkages should prevent the toxicity effect, associated to phosphorothioate linkages, by increasing the affinity for the target mRNA (2).

Partial PS ODNs have been used to control blood pressure in normotensive (WKY) and hypertensive (SHR) rats. Molecules were injected intracerebroventricularly (i.c.v., right lateral ventricle) in freely moving animals. The antisense ODN lowered the mean arterial pressure (MAP) of SHRs 24 hours (-43 mmHg±10) and 48 hours (-30 mmHg±13) after injection, while the control ODN molecule had no significant effects. The observed decrease of blood pressure was due to a specific inhibition of AT_1 receptor gene expression, since the level of its mRNA, monitored by reverse transcription (RT)- polymerase chain reaction (PCR), was significantly reduced by antisense molecule (-40%), compared to sense one. In normotensive rats no effect on MAP by antisense treatment have been observed, while at molecular level the AT_1 receptor gene expression is reduced (-40%). It is known that SHRs have an enhanced basal activity of the central renin-angiotensin system that induces an increase in central sympathetic outflow. Instead in WKY rats the central sympathetic outflow is not conditioned by the enhanced activity of brain renin-angiotensin system. The hypothesis to explain these findings is that in normotensive rats, although partial PS ODN reduces

the AT_1 mRNA level, this will not result in a modification of the sympathetic outflow and no change in MAP level would be observed.

References

1. R. Gyurko, D. Wielbo and M.I. Phillips. *Antisense inhibition of AT_1 receptor mRNA and angiotensinogen mRNA in the brain of spontaneously hypertensive rats reduces hypertension of neurogenic origin*. Regulatory Peptides 49 167-174 (1993).

2. U. Galderisi, G. Di Bernardo, M.A.B. Melone, G. Galano, A. Cascino, A. Giordano and M. Cipollaro. *Antisense inhibitory effect: a comparison between 3'-partial and full phosphorothioate antisense oligonucleotides*. J. Cell. Biochem. 74(1) 31-37 (1999).
