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EFFECT OF THE COMBINATION OF NCX 4016, ASPIRIN (ASA) AND CLOPIDOGREL IN A MODEL OF PULMONARY THROMBOEMBOLISM IN MICE.

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The combination between a thienopyridine and aspirin represents the treatment or choice for patients undergoing coronary angioplasty with stent implantation. Moreover, recent trial results have shown that the combination of clopidogrel and ASA in unstable angina is superior to aspirin alone, however at the price of enhanced bleeding. NCX 4016, an aspirin derivative releasing nitric oxide, exerts an antithrombotic activity superior to aspirin in some animal models and reduces the degree of restenosis after arterial injury in hypercholesterolemic mice and in adult rats.
We have compared the effect of ASA, NCX 4016, clopidogrel and various combinations of them on platelet pulmonary thromboembolism and on bleeding in mice. Drugs were administered orally once a day for five days. The studies were carried out one our after the last oral administration.
Results:

	Mortality (%)	Occluded Lung vessels (%)	Platelets ($x10^3/\mu l$)	Plasma NO2/NO3 (μM)	Serum TxB2 (ng/ml)	Bleeding time (sec)
Control mice	80	92.5±4.1	87.6±12.62	14 ± 2.5	101 ± 19.7	202 ± 10.5
ASA 30 mg/kg	75	96.6±3.34	70.85x12.59	10.6 ± 4	8 ± 2.9*	410 ± 114*
NCX 4016 60 mg/kg	37*	64.32±12.87	239.71±52.42	36.7 ± 3.27*	28.6 ± 12*	415 ± 95*
Clopidogrel 0.5 mg/kg	78	71.66±10.85	173.71±13.03	17.8 ± 6	78.2±20.07	451.2 ± 72*
ASA + Clopidogrel	53	68.41±7.86	162.71±24	-	5.25 ± 2*	800 ± 68*
NCX + Clopidogrel	41*	45.88±7.64	324±40.9	32 ± 8.5*	19.6 ± 9.2*	484 ± 90*#
ASA+NCX+Clopidogrel	40*	49.24±7.9	276.29±29.6	36.2 ± 13.3*	5.8 ± 2.5*	733 ± 87*

*p>0.05 vs control mice; #p<0.05 vs ASA+Clopidogrel

The ex vivo platelet aggregation induced by ADP (0.8μM) was inhibited or significantly reduced in all animals treated with clopidogrel alone or in combination. Arachidonic acid (AA)(0.2 mM)-induced platelet aggregation was abolished in all animals treated aspirin. NCX 4016 inhibited AA-induced aggregation and it also reduced significantly U46619-induced aggregation.
The combination of aspirin and clopidogrel exerts a stronger antithrombotic protection as compared with the single drugs, although associated with a striking prolongation of the bleeding time. On the other hand, the combination of NCX 4016 and clopidogrel exerts an even greater protection against collagen + epinephrine induced thromboembolism associated with a lesser prolongation of the bleeding time. Addition of NCX 4016 to the combination ASA+clopidogrel does not further increase antithrombotic protection but it does not enhance bleeding either. In therapeutic procedures of revascularization associated with a risk of restenosis in which ASA + clopidogrel is the first choice therapy, such as angioplasty with stenting, an associate use of NCX 4016 could be hypothesized.