**BIOLOGICAL ACTIVITY**

**Description**
E 64c is a derivative of naturally occurring epoxide inhibitor of cysteine proteases, a Calcium-activated neutral protease (CANP) inhibitor and a very weak irreversible cathepsin C inhibitor.

**IC$_{50}$ & Target**
Cysteine proteases$^{[1]}$, CANP$^{[2]}$, Cathepsin C$^{[3]}$.

**In Vitro**
E-64c, a derivative of naturally occurring epoxide inhibitor of cysteine proteases, with papain; especially with regard to the hydrogen bonding and hydrophobic interactions of the ligands with conserved residues in the catalytic binding site$^{[1]}$. E 64c ($k_2/K_i=140\pm5M^{-1}s^{-1}$) is demonstrated to be a lead structure for the development of irreversible cathepsin C inhibitors$^{[3]}$.

**In Vivo**
The t-1/2 of plasma E-64c is 0.48 hours. The hemodynamic effects of E-64c are absent at this dose. Using two way analysis of variance, the effects of reperfusion (p=0.0016) or E-64c (p=0.0226) per se on infarct size are significant. In comparing Group A with Group B and Group C with Group D, the depletion of CPK in the E-64c treated groups (Groups A and C) is slightly less than in the vehicle-injected groups (Groups B and D). The insufficient effect of E-64c alone may be explained by the early administration and relatively short t-1/2. Since the effectiveness of NCO-700 has been established$^{[6,7]}$, our findings might indicate a small but beneficial effect of E-64c on infarct size and CPK content$^{[2]}$.

**PROTOCOL**

**Animal Administration**$^{[2]}$

Studies are carried out in 83 mongrel dogs with a mean weight of 11.2kg. They are anesthetized with intravenous sodium thiamylal (7mg/kg). An intravenous bolus of E-64c (100mg/kg), dissolved in saturated sodium bicarbonate, is administered immediately before the occlusion and after reperfusion in Group A (n=17), whereas Group B (n=17) receive only the vehicle solution at these times. In the remaining 49 dogs (Groups C and D), the LAD is permanently ligated at the same level and an intravenous bolus of either Loxistatin acid (100mg/kg) (Group C; n=24) or vehicle only (Group D; n=25) is given immediately before and 1 hour after the ligation. The dose of E-64c is designed for its possible use in clinical practice and the estimated intramyocardial Loxistatin acid molecular concentration is 1,000 times that of total mCANP$^{[2]}$.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.
REFERENCES

