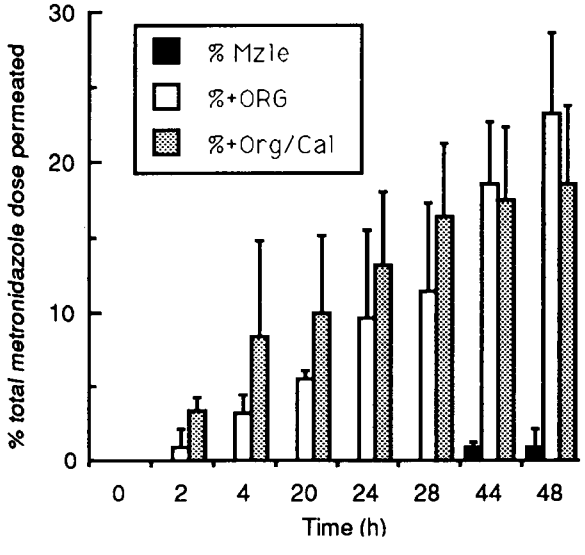
MEDICINAL LEECH HYALURONIDASE AS A POTENTIAL SKIN PENETRATION ENHANCER: IN-VITRO PERMEATION STUDIES

D.G.Williams, J. Hadgraft, Welsh School of Pharmacy, U.W.C.C., Cardiff CF1 3XF
and R.T.Sawyer, C. Powell-Jones, Biopharm (UK) Ltd., Hendy, Dyfed SA4 1XB

We have investigated the effects of the medicinal leech enzymes Orgelase and Calonase on the permeation of a model non-polar drug, metronidazole (2- {2-methyl-5-nitroimidazoyl-1-yl} ethanol) through excised human cadaver skin.
It has been discovered that the common European leech, Hirudo medicinalis, when feeding secretes in addition to the anticoagulant hirudin, a specific hyaluronidase, Orgelase, and a collagenase, Calonase. Orgelase acts in-vivo by causing the anticoagulant to spread across the skin (Sawyer 1986). Being specific, it binds to hyaluronic acid- a polysaccharide that secures cells together. It also has a ß-glucuronidase type specificity which may be characteristic of jawed leeches.
Full thickness female caucasian skin was obtained post mortem, dissected and clamped dermal side downwards in Franz type glass diffusion cells (Franz 1975). The receptor compartments were filled with iso-osmotic pH 7.4 phosphate buffered saline, and maintained at a temperature of 37oC (+/-2oC). The skin and receptor solution were left to equilibrate for an hour before any pretreatment. Orgelase and Calonase were reconstituted in appropriate buffer solutions, and the skin pretreated with a specific volume of the enzyme, control cells pretreated with buffer alone. The metronidazole dose was applied to the skin surface one hour after pretreatment. The passage of drug from the stratum corneum side of the membrane to the receptor fluid was monitored by sampling at regular intervals from a receptor port. Skin permeation was studied over a period of 48 hours.



Results seen in the figure, show that Orgelase causes an initial flux of metronidazole, followed by up to a twentyfold increase in flux through the skin as compared with the controls. This is probably due to the enzyme being at its most active during the first two hours after reconstitution. Orgelase may actively dissociate the cells in the skin, allowing flux of metronidazole across the skin tissue. Calonase alone, does increase flux of metronidazole across the skin-compared to the controls, however these results were not as significant as those seen with skin pretreated with Orgelase alone.

From results obtained, and due to the unique mechanism of action and known substrate specificity of the leech enzymes, lead us to believe that Orgelase has potential as a skin penetration enhancer.

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