Additive Effects of Collagen and Adrenaline on Platelet Aggregation in 96-well Plates

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Platelet aggregation routinely measured by light transmission techniques (Born and Cross, 1962) has been developed in a simple 96-well plates (Moran *et al*, 2006). This method allows testing of many aggregatory responses over an identical short time period. When a pair of platelet agonists in low concentration is added together or in sequence to platelet-rich plasma, the effect of each other in platelet response is enhanced (Steen *et al*, 1988). We have evaluated the effects of combined collagen and adrenaline in platelet-rich plasma using 96-well plate method.

Human blood was collected by venepuncture into tri-sodium citrate (3.8% w/v final) and centrifuged to obtain platelet rich plasma (PRP). The PRP was then added to the wells of 96-well plates in the presence or absence of collagen, adrenaline or combination of collagen and adrenaline. Plates were then immediately placed in a 96-well plate reader and absorbance determined at 595nm every 15s for 16min with vigorous shaking. Changes in absorbance were converted to % aggregation by reference to the absorbance of PRP and platelet-poor plasma.

Aggregatory responses to pair agonists were enhanced compared with collagen or adrenaline alone. For example at 4min, percentage of aggregation by combined 1µg/ml collagen and 10^{-7} M adrenaline was 56±16% compared with 1µg/ml collagen alone, 27±12% or 10^{-7} M adrenaline alone, 22±5%. At 8 min, combinations of 10^{-7} M adrenaline with 0.1, 0.3 and 1 µg/ml collagen enhanced the aggregation to 61±12%, 64±14 and 79±9 respectively as compared with collagen alone (0.1µg/ml, 34±10; 0.3µg/ml, 42±14; 1µg/ml, 57±7) or 10^{-7} M adrenaline, 46±9%.

By using 96-well plate format, we demonstrated the additive effects of the combinations of low collagen and adrenaline concentrations on platelet aggregation. In conclusion, this findings could be useful since low concentrations of several agonists may mimic the conditions under which thrombosis occur in vivo.

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