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Perfusion of rabbit hearts with pig blood results in complement mediated hyperacute xenograft rejection

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Investigation of hyperacute rejection of discordant xenografts has been hampered by the lack of a model for the study of rapid time course events. In vivo models are unsuitable for observation of early rejection processes and, along with most ex-vivo perfusion preparations, are insensitive since no functional demand is placed on the organ which may have undergone extensive damage whilst still appearing viable. For this reason a blood perfused isolated working heart preparation has been developed [1]. With left atrial and left ventricular loading the heart performs measurable work as it ejects into a mock circulation with both afterload and compliance components. When cardiac function is compromised the heart is no longer able to eject against the fixed afterload and both cardiac output and coronary circulation cease with resultant organ failure. The model is thus highly sensitive to minimal organ damage and has an easily identifiable endpoint. In the present study, we used this preparation to study the discordant species combination of rabbit hearts perfused with pig blood.

Key words: Blood perfused isolated working heart preparation – Rabbit hearts – Rabbit blood – Pig blood

Methods

Hearts of 1.7 kg New Zealand White rabbits were perfused with either rabbit blood collected into heparin (6500 units/l) and with the haematocrit reduced to 25%, or similarly collected and treated pig blood either unmodified or complement depleted with cobra venom factor (CoF). There were four hearts in each perfusion group. Hearts were perfused until functional damage caused their failure. A log-rank analysis of survival was performed.

Complement depletion was achieved by the addition of 10 µg purified CoF [2] to 240 mls plasma. Lytic pig anti-rabbit antibody (ARA) titres were measured before and after perfusion by a haemolytic method [1]. Complement classical pathway activity was measured with a CH50 technique [2]. Hearts were examined after failure by conventional and immunohistological methods (IgG, IgM, C3).

Results

Rabbit hearts perfused with rabbit blood survived for a median time of 271 min. Perfusion with unmodified pig blood resulted in organ rejection at a median time of 13 min ($P < 0.001$). Total complement haemolytic activity (CH50) and ARA titre was unchanged during perfusion. Conventional histology revealed lymphoid and neutrophil cell infiltrates and immunofluorescence showed interstitial IgG and endothelial deposits of IgM and C3. Perfusion with pig blood treated with CoF (complement activity completely removed) produced survival of a median time of 175 min ($P < 0.001$). The myocardium remained normal and IgG and IgM were deposited but no C3 was seen. Organ survival is summarised in Fig. 1.

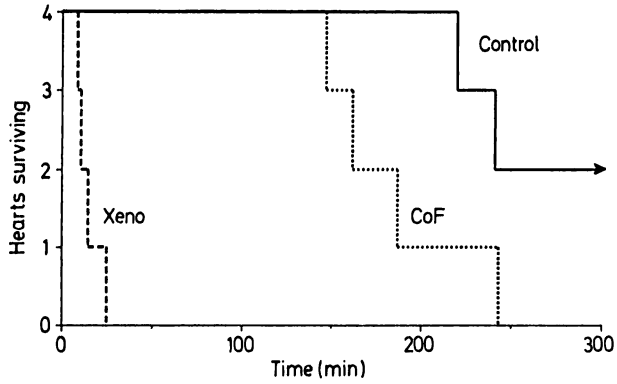

Fig. 1. Survival of rabbit hearts perfused with rabbit blood (auto), pig blood (xeno) and complement depleted pig blood (CoF)

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Discussion

A sensitive perfusion model for the investigation of rapid time course events has been developed and has been used to simulate hyperacute discordant rejection of rabbit hearts by pig blood. Prevention of rejection by complement inactivation and the detection of C3 on the perfused myocardium demonstrated the central role of complement in the process. No conclusion could be made, however, concerning the relative importance of the classical and alternative pathways of complement. Further analysis of rejection in this species combination is prevented by the lack of immunological reagents for the investigation of pig blood. The sensitivity of this technique will permit detailed analysis of the components of rejection of rabbit hearts by human blood where a wider range of immunological tools is available.

References

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