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Title: Investigating the pathology of cobalt-induced pseudo-tumour formation
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Introduction:
Approximately five percent of patients who get a hip replaced develop tumour-like growths. These growths are termed pseudo-tumours because of their structural similarly to cancerous tumours; they both have a hypoxic centre, where there is a lack of oxygen, and a vascularised exterior. However, cancerous tumours grow uncontrollably due to mutations in the DNA whereas pseudo-tumours do not appear to carry any mutations. This makes the pseudo-tumours particularly interesting, because they could indicate factors that drive the growth of a tumour which do not stem from changes in the DNA.

Interestingly, pseudo-tumour formation only occurs to those who have failing metal hips. These metal hips are often made of a cobalt alloy, and the daily grinding can cause micro-flakes of metal to be embedded in the surrounding tissue. So the main question is how does a failing hip cause this tumour-like growth? What proteins and factors are involved?

The hypothesis is that pseudo-tumour formation is due to the establishment of a pro-tumour characteristic in normal cells as a result of cobalt toxicity. Cobalt is known act as a hypoxia mimetic and increases the presence protein called HIF1a, which is a major cancer growth promoter. HIF1a generates VEGF which directs the formation of new blood vessels and allows for cell survival under stressful conditions.

We propose to investigate whether cobalt toxicity in pseudo-tumours results in HIF-1a expression that might explain the formation of these growths in hip-replacement patients and also provide insight into the role of HIF-1a in cancerous tumour formation.

Aim:
The aim of this project was to characterise the tissue from these pseudo-tumours, which was split into two parts. 1) To determine what method would provide the best way to characterise the protein expressed in the tissue and 2) to use this method to determine whether HIF1a and any of the proteins under its control are present.

Method:
Prior to any technical analysis, the tissue was ground up and DNA analysis to was used to determine the cellularity of the tissue (how much of the tissue was cells). Once this was complete several techniques were used to examine the presence of various factors in the tissue. These included western blotting (to visualise the presence of HIF1a and other proteins), ELISA (to determine the concentration of VEGF), high pressure liquid chromatography (HPLC; to measure the vitamin C levels in the tissue) and immunohistochemistry (to determine the organisation of the tissue, what types of cells are present, and the localisation certain proteins).

Results:

DNA analysis showed a content of 0.88ug per milligram of tissue (highest content in any of the samples). This was very low when compared to other tissues (e.g. cancerous tissues) that have used the same method and that have approximately three times more DNA.
Looking at the tissue under the microscope it was evident this was due to the high fat and collagen content, in conjunction with a high volume of dead tissue (or necrotic tissue). This made the use of western blotting as an analytical tool inappropriate. More sensitive analyses (HPLC and ELISA) were able to determine the concentration of vitamin C and VEGF, respectively. The vitamin C content varied from no detectable levels to 6.34ug/100mg wet weight. This is not uncommon considering muscle cells have the lowest levels of vitamin C, which in itself is dependent on diet. The VEGF ranged from 0.44pg/ug to 3.9pg/ug, which is in the range for 'normal' tissue.

Therefore, immunohistochemistry is the most effective method to study HIF1a in this tissue. When stained with a morphological stain, the masses of fat and infiltrating immune cells become apparent. Using this method, we were able to demonstrate the presence of HIF-1a and to investigate the vascularity, immune cell infiltration, and the properties of the fat bodies.

Conclusion:
In this study, it has become evident that the best analytical tool for this tissue is immunohistochemistry, and this has demonstrated the presence of HIF-1a, a known tumour growth promoter, in the pseudo-tumour tissue. This information, together with the analysis of tumour-like morphology and the expression of downstream HIF proteins provides evidence that the characteristics of the pseudo-tumours are similar to cancerous tumours. This will be the subject of a future publication.