199g Evaluation of the Angiogenic Capacity of Small-Size Oligosaccharides of Hyaluronic Acid

Harold Castano, Paul L. DeAngelis, and Vassilios I. Sikavitsas

Oxygen and nutrients are necessary for the growth, maintenance and reproduction of cells, tissues and organs in health and disease. Tissue engineering has proposed strategies to generate tissues by combining biological and engineering sciences in order to replace lost or malfunctioning organs. One drawback in tissue engineering has been the delivery of oxygen and nutrients to growing tissues. Thus, creating vascular supply is of paramount importance for tissue engineering since it is impossible to grow tissues further than 100µm in 3D without blood vessels. One strategy used to stimulate angiogenesis — the formation of vascular supply from already formed blood vessels— has been the use of angiogenic factors. Hyaluronic acid (HA) is a glycosaminoglycan found mainly in the lubricating fluid of joints, cartilage, bone, vitreous humor. HA has become particularly interesting since it possesses a dual effect on blood vessel formation: in the natural form, it has been reported to be anti-angiogenic (although further research is needed to confirm that), but when it breaks down, it shows a strong angiogenic effect. Studies using HA species derived after enzymatic digestion of the natural biopolymer, (a process that makes it difficult to identify if there is a particular size of oligosaccharides that is predominantly responsible for the angiogenic effect). This study evaluated oligosaccharides of HA of a previously-determined size made by chemical synthesis on the chick embryo chorioallantoic membrane (CAM) model. The chorioallantoic membrane is a highly vascularized tissue formed during the third to fourth day of life of the embryo by the fusion of the chorion (a tissue derived from the ectoderm) and the allantois (a tissue derived from the endoderm) and used for gas exchange during the embryogenesis of birds. For this purpose, Leghorn eggs were incubated in a hatching incubator for three days, cracked out, deposited on Petri dishes and returned to a controlled incubator under 38°C, 5% CO_2, and 70% relative humidity. Oligosaccharides of hyaluronic acid of 4, 8, 12, 18, 20 and 22 disaccharides were used during this study. On day eight, oligosaccharides were dissolved in double distilled water, and ten micrograms of each one were placed on one side of the plastic cover slip and turned over on top of the CAM. Double distilled water deposited on the plastic cover slips was used as a control. Each group consisted of a minimum of 15 eggs ($n = 15 – 25$) due to the inherent noise of the experimental model. On day eleven, pictures of the CAM with cover slips in place were taken and recorded for further analysis. Three analyses methods were chosen to evaluate the data: 1) the angiogenic index, 2) the vascular density index, and 3) the fractional image. The first method compares the place of angiogenesis with the surrounding tissue and assigns a score to the findings. The scoring is later normalized by dividing the assigned score by the maximum score attainable. The second method measures the number of blood vessels that cross over a set of three concentric circles separated by a known distance; this method is used because the length of blood vessels relates to the angiogenic response. The third method measures the area of blood vessels grown around the location where the oligosaccharides of HA were applied. The first two methods were performed in a double blind fashion. All CAMs showed some degree of angiogenesis. This was expected since the CAM is still under formation when the oligosaccharides of HA were applied. However, an enhanced response was observed with the application of oligosaccharides of size 20 in all three tests compared to the controls. In addition, on the fractional vessel area test, a statistically significant response was observed for the oligosaccharide of size 20 compared with all other groups of oligosaccharides. We concluded that the oligosaccharides of HA in the range of 4 to 22 disaccharides showed a better angiogenic response than the controls, and that oligosaccharides of 20 disaccharides have the best angiogenic effect on blood vessel formation on the chorioallantoic membrane. The better response obtained with the size 20 oligosaccharide suggests that spatial conformation may play a role in the mechanism of angiogenesis. An alternative in vivo model to evaluate angiogenesis of HA oligos entrapped in hydrogels and injected subcutaneously in rats is currently under evaluation. The use of the much hardier oligomer of HA (HA-20) as an angiogenic agent is expected to allow novel and much more flexible tissue engineering strategies compared to the traditionally used Vascular Endothelial Growth Factor.