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Iwona Becka, Anna Hotowya, Ewa Sawosza, Marta Grodzikb, Mateusz Wierzbickia, Marta Kutwina, Sławomir Jaworski a & André Chwalibogb

a Division of Nanobiotechnology, Warsaw University of Life Sciences, Warsaw, Poland
b Department of Veterinary Clinical and Animals Sciences, University of Copenhagen, Frederiksberg, Denmark

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Effect of silver nanoparticles and hydroxyproline, administered in ovo, on the development of blood vessels and cartilage collagen structure in chicken embryos

Iwona Beck\textsuperscript{a}, Anna Hotowy\textsuperscript{a}, Ewa Sawosz\textsuperscript{a}, Marta Grodzik\textsuperscript{a}, Mateusz Wierzbicki\textsuperscript{a}, Marta Kutwin\textsuperscript{a}, Sławomir Jaworski\textsuperscript{a} and André Chwalibog\textsuperscript{b*}

\textsuperscript{a}Division of Nanobiotechnology, Warsaw University of Life Sciences, Warsaw, Poland; \textsuperscript{b}Department of Veterinary Clinical and Animals Sciences, University of Copenhagen, Frederiksberg, Denmark

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It has been considered that concentrations of certain amino acids in the egg are not sufficient to fully support embryonic development of modern broilers. In this study we evaluated embryo growth and development with particular emphasis on one of the major components of connective tissue, collagen. Experiments were performed on Ross 308 chicken embryos from 160 fertilised eggs. Experimental solutions of silver nanoparticles (Ag), hydroxyproline solution (Hyp) and a complex of silver nanoparticles with hydroxyproline (AgHyp) were injected into albumen, and embryos were incubated until day 20. An assessment of the mass of embryo and selected organs was carried out followed by measurements of the expression of the key signalling factors’ fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor-A (VEGF-A). Finally, an evaluation of collagen microstructure using scanning electron microscopy was performed. Our results clearly indicate that Hyp, Ag and AgHyp administered in ovo to chicken embryos did not harm embryos. Comparing to the control group, Hyp, Ag and the AgHyp complex significantly upregulated expression of the FGF-2 at the mRNA and protein levels. Moreover, Hyp, Ag and, in particular, the complex of AgHyp significantly increased blood vessel size, cartilage collagen fibre lattice size and bundle thickness. The general conclusion from this study is that AgHyp treatment may help to build a stronger and longer lasting form of collagen fibres.

Keywords: chickens; collagen; connective tissue; embryo development; hydroxyproline; nanoparticles; silver

1. Introduction

The growth and development of embryos from new fast-growing broiler chicken breeds can be compromised due to inadequate accumulation of nutrients, such as amino acids, within the eggs. This can result in shortages of signalling factors, fundamental proteins and nutrients, which can hamper optimal development of collagen, the most common building block of embryonic tissue. Except maternal nutrition, the only way to supply compounds needed for optimal embryonic and post-hatch development can be in ovo administration to the chicken embryo (Noy and Uni 2010; Sawosz et al. 2012; Zielinska et al. 2012). The quality of muscle structure, proper growth and the formation of all tissues and organs are related to the correct development of the connective tissue. This type of tissue is present in almost all structures of a living organism, with a prime example being the embedding of blood vessels in connective tissue (Chvapil 1967; Gay and Miller

*Corresponding author. Email: ach@sund.ku.dk

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In turn, its correct development is highly dependent on the availability of proline and hydroxyproline, the predominant amino acids of collagen. As with all proteins, the amino acid distribution determines the macromolecular properties and in collagen regions containing the standard Gly-Pro-Hyp (glycine-proline-hydroxyproline) amino acid motif form rigid helical structures, while those regions lacking hydroxyproline and proline residues are more flexible and can conform to store energy (Silver et al. 2003). Proline is necessary for protein synthesis and for redox stabilisation by the shuttle of redox equivalents between the cytosol and mitochondria. Hydroxyproline as an analogue of proline regulates growth, ROS (reactive oxygen species) generation and apoptosis (Berger et al. 2001). Furthermore, when proline levels are low, hydroxyproline can be metabolised, which will maintain an even distribution of both proline and hydroxyproline for collagen synthesis.

Two major families of growth factors are involved in collagen development: fibroblast growth factors (FGF) and vascular endothelial growth factors (VEGF). They have different effects on endothelial cells but intercommunicate and coordinate their actions (Presta et al. 2005). FGF are broad-range morphogens that play significant roles during embryonic development and also carry out important roles in mesoderm induction and the development of the brain, blood vessels and lungs (Cross and Claesson-Welsh 2001). Overexpression of FGF-1 or FGF-2 in mice results in increased vascular density and branching (Javerzat et al. 2002), with FGF-2 also having an important function in the physical organisation of embryonic cells into tube-like structures (Prior et al. 2004). Furthermore, Imamura et al. (2007) has linked FGF-2 to a downregulation of Type I collagen but the upregulation of Type III collagen synthesis. VEGF is a sub-family of the platelet-derived growth factor family playing significant role in blood vessel formation (Klagsbrun and D’Amore 1996). VEGFs are important signalling proteins involved in angiogenesis. Excessive VEGF can lead to abnormal vascular patterning and an excessive fusion of blood vessels. The most important member of this sub-family is VEGF-A, which has four alternately spliced isoforms, including soluble VEGF\(_{121}\) and matrix-bound VEGF\(_{189}\). VEGF\(_{121}\) increases endothelial cell mitosis while VEGF\(_{189}\) increases the migration of endothelial cells, which upregulates the permeability of the basement membrane and facilitates pore formation (fenestrae) in endothelial cells (Wang et al. 2008).

Nano-nutrition is a new methodology based on the use of nanoparticles as potential vehicles to transport nutrients to specific targets, thereby improving their bioavailability. Silver belongs to the group of noble metals and silver nanoparticles have affinity for primary amines (protein N-termini and Lys side chains), exhibit tropism for connective tissue and also have anti-microbial properties. Thus, they may be useful as particles that can transport amino acids to tissues and their cells. It has also been reported that silver nanoparticles can kill microbes more rapidly and thoroughly than their cationic forms (Rai et al. 2009). The significance of using nanoparticles is due to the dramatic increase in surface area in relation to their size. Moreover, their nanoscale (1–100 nm) also allows them to pass through biological barriers (e.g. to the brain and eye); however, nanoparticle bio-distribution cannot be predicted once the solution has entered the body, which can be affected by reactions with proteins and the mononuclear phagocyte system (Sanvicens and Marco 2008). Based on the available literature and previous research, we hypothesise that providing chicken embryos with the complex of silver nanoparticles and hydroxyproline in ovo may improve the morphology and function of connective tissue and could improve the embryo’s growth and development.
2. Material and methods

2.1. Preparation of experimental factors

The colloidal nanosilver solution (50 mg per litre deionised water, 99.9999% purity) was obtained from Nano-Tech Poland Ltd (Warsaw, Poland). The average particle size was 3.5 nm, accounting for more than 80% of all particles. The average particle surface area was $2.827 \cdot 10^{-13}$ cm$^2$. The pH range was 7.1–8.1 and the redox potential was 0.1 mV. High purity (>99%) hydroxyproline (trans-4-hydroxy-L-proline, C$_5$H$_9$NO) was purchased as a powder from Sigma-Aldrich, USA and mixed with deionised water to form a 0.7% aqueous solution. Prior to use, all experimental factors (0.7% hydroxyproline, 50 mg/l silver nanoparticle colloid and a combination of 0.7% hydroxyproline with 50 mg/l silver nanoparticle colloid) were sonicated for 30 min at 37°C in a Sonorex Super RK 514H ultrasonic bath (Bandelin, Germany).

2.2. Injection of chicken eggs with experimental factors

Fertilised eggs were obtained from a specialised farm (Lydia and Henry Malec; Dębówka, Poland). The laying flock consisted of 30–42-week-old Ross 308 hens. All eggs were stored at 14–16°C for 4 d prior to use. Experiments were performed on 160 fertilised eggs, which were weighed, labelled and randomly distributed into four groups (40 eggs in each group): (1) the untreated control group (C), (2) the group treated with 0.7% hydroxyproline (Hyp), (3) the group treated with the silver nanoparticle colloid (Ag) at 50 mg/l and (4) the group treated with a mixture of 0.7% hydroxyproline and the silver nanoparticle colloid at 50 mg/l (AgHyp). Prior to incubation, the eggs were disinfected with 75% ethyl alcohol solution followed by sterilisation through exposing the surface to ultraviolet radiation for 50 s. A sterile scalpel was then used to make a small hole one-third the length of the egg from the pointed end (Figure 1). A sterile disposable tuberculin needle was used next to inject 300 µl of experimental solutions through the hole into the albumen. The hole was sealed with 25 mm hypoallergenic Polopore tape (Viscoplast 3M, USA). All eggs were placed in an ALMD-1N3-7 incubator (FHU Walenski, Gostyn, Poland), under standard conditions.

2.3. Necropsy of chicken embryos

At the age of 20 d, the embryo was removed from the egg, weighed and then decapitated. The liver, heart (with large blood vessels attached) and spleen were collected. Each organ

![Figure 1](image_url)
was weighed, wrapped in foil, placed in a separately labelled plastic bag and frozen at
−80°C. Sternum cartilage was excised from each test animal, wrapped in foil, placed in
separately labelled bags and frozen at −80°C. Left pectoral muscle fragments were
extracted and stored in RNAlater (Invitrogen, USA) at 8°C for subsequent q-PCR
analysis. Right pectoral muscle fragments were extracted from control and treated
embryos and then stored in liquid nitrogen at −80°C for subsequent ELISA analysis.

2.4. q-PCR analysis of gene expression at the mRNA level

RNA was isolated using the SV Total RNA isolation System (Promega, USA) according
to the manufacturer’s protocol. Total RNA concentration was measured using a NanoDrop
ND 1000 Spectrophotometer (NanoDrop products, USA). Total RNA (2 μg) was reverse
transcribed using reverse transcriptase (Promega, USA), oligo-dT and random primers,
(TAG Copenhagen A/S Symbion, DK) after which real-time PCR was performed with
cDNA and gene-specific primer pairs (TAG Copenhagen A/S Symbion, DK) mixed with
LightCycler® 480 SYBR Green I Master (Roche, CH) in a LightCycler® 480 system
(Roche, CH) (Table 1). The samples were first denatured for 5 min at 95°C and then
amplified using 45 cycles of 10 s at 95°C (denaturation), 10 s at 60–62°C (annealing) and
9 s at 72°C (elongation) followed by quantitation. For each cDNA the reaction was
performed in triplicate. For all analyses, relative quantification was applied and cycling
reports and melting curves were evaluated. Actin beta (ACTB) was used as a house-
keeping gene. All reactions were performed in triplicate.

2.5. ELISA analysis of gene expression at the protein level

The sample was placed in chilled RIPA buffer (10 mM Tris pH 7.5, 150 mM NaCl, 0.5%
sodium deoxycholate, PMSF 0.1 mg/ml, 1% NP40, 0.1% SDS, 1 mM sodium orthovan-
date, leupeptin 10 μg/ml, aprotinin 0.05 mg/ml and pepstatin 5 μg/ml) and homogenised
on ice using a Polytron PT-MR 2100 (Kinematica, Switzerland) mixer. The homogenate
was left on ice for 30 min, followed by centrifugation at 10500 g and 4°C for 20 min.
The supernatant was aliquoted in Eppendorf tubes to avoid frequent freeze/thaw cycles and
frozen at −20°C. Each aliquot has been used for determining protein concentration (Total
Protein Kit, Micro Lowry, Peterson’s Modification, Sigma-Aldrich, USA) and for ELISA
test. ELISA was carried out according to the manufacturer’s instructions using a set of
reagents supplied with the following commercial kits: Gallinaceous Fibroblast Growth

Table 1. Primers used for q-PCR reaction.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene ID*</th>
<th>Size</th>
<th>Primer sequence (5’ to 3’)</th>
</tr>
</thead>
</table>
| ACTB†       | 396526  | ca. 169 bp | Forward: GTC CAC CTT CCA GCA GAT
Reverse: ATA AAG CCA TGC CAA TCT CG |
| FGF-2‡      | 396413  | ca. 151 bp | Forward: GGC ACT GAA ATG TGC AAC AG
Reverse: TCC AGG TCC AGT TTT TGG TC |
| VEGF-A◊     | 395909  | ca. 194 bp | Forward: TGA GGG CCT AGA ATG TGG CT
Reverse: TTC TTT GAC CCT TCC CCT TT |

Notes: *NCBI resources; †ACTB, Actin beta; ‡FGF-2, Fibroblasts growth factor-2; ◊VEGF-A, Vascular endothelial growth factor-A.
Reagents and plates were prepared according to the manufacturer’s instructions. The level of absorption was measured in an Infinite M200 microplate reader (TECAN, DE) at 450 nm. All samples were measured in duplicate.

2.6. **Scanning electron microscopy and image analysis**

Frozen sternum cartilage and heart blood vessels were cleanly cut with a scalpel into 1 mm thick sections on frozen pieces of wax. While still frozen, a sample section was placed on a Peltier table within a standard cooled microscope column (at −5°C for blood vessels and −10°C for cartilage) in the sample chamber under high vacuum (10⁻⁵ Torr). These were then observed with a scanning electron microscope (EM) QUANTA 200 (FEI, Japan), Olympus, Warsaw, Poland.

Scanning EM image measurements were performed using a simple freeware drawing tool (paint.net, USA) to superimpose rectangles onto the scanning EM images. The tool reported the bound area size in pixels and as images were all taken at 1000× magnification, the pixel count provided a good relative comparison between tissue samples. To determine collagen lattice unit area, the largest rectangular areas enclosed by a four-sided set of joined fibre bundles were selected and then the next biggest areas were identified. This was repeated until up to 14 rectangles per scanning EM photo were found. To estimate collagen fibre bundle thickness, the 14 or less superimposed rectangles per scanning EM image that were created for area calculations were reused. To avoid overlapping measurements, the thinnest points at the top and right sides of each rectangle were examined. At these points, a one-pixel wide rectangle was drawn perpendicularly across the fibre bundle. The area reported by paint.net was then the minimum thickness of respective bundle side in pixels.

2.7. **Statistical methods**

Statistical analysis was performed using two-way ANOVA. To determine the difference between the groups, the Duncan’s multiple range test was applied. If any significant interaction was observed, a follow-up analysis was carried out using monofactorial ANOVA. The statistical software package that was used was Statgraphic 4.1 Plus (Manugistics Corp, USA). Values of $p \leq 0.05$ were considered significant.

3. **Results**

3.1. **Embryo body and organ mass**

Embryo growth and development was evaluated by an assessment of the embryo mass and the mass of the selected organs (Table 2). Regarding the relative mass of the embryo compared to the egg mass, no significant differences were observed between groups and no significant interaction existed. The ratio of heart mass to embryo mass was significantly different between Groups C and Hyp. The relative mass of livers and spleens was also not significantly influenced by treatments.

3.2. **Molecular effects**

Results concerning gene expression at the mRNA level are summarised in Table 3. The FGF-2 and VEGF-A mRNA expressions were lowest for the Control group. The expression
of FGF-2 mRNA was highest in Groups Hyp and Ag ($p < 0.05$) and Group AgHyp showed a tendency ($p < 0.1$) of a higher expression than Group C. Furthermore, significant interactions between Hyp and Ag treatments were observed. The expression of VEGF-A
mRNA was significantly highest in Group Ag and no significant differences between Groups C, Hyp and AgHyp were observed.

When focussing on protein levels (Table 3), FGF-2 levels were the lowest in the Control group, while Groups Hyp and AgHyp had the highest levels, which were significantly different from Group C. VEGF-A protein levels were not significantly different between treatments.

### 3.3. Blood vessel and cartilage development

Samples of blood vessels from 20-d-old embryos were visually inspected using a scanning EM (Figure 2), which indicated that the blood vessels (inner wall, between vessels or outer wall) did not appear to be affected by general structural trends.

While the Control group did not have uniform collagen bundles, Group Hyp had a greater number of collagen bundles that formed polygonal structures, although both were not uniform in size or directionally oriented. Group Ag introduced a lattice-like organisation to the structures with long, straight fibre bundles and apparent directionality, although there was still no size uniformity. However, Group AgHyp had much larger and thicker lattice-like structures, with a more uniform size and directional orientation. Furthermore, these observations were also consistent with our other examinations. The results of the average area of a rectangular collagen lattice unit (Table 4) demonstrate a significantly larger lattice area in Group AgHyp than in the other groups. In addition, the results of the collagen bundle thicknesses also show that bundles appear to be much thicker in Group AgHyp than in Groups C, Hyp and Ag.

<table>
<thead>
<tr>
<th>Group C</th>
<th>Group Hyp</th>
<th>Group Ag</th>
<th>Group AgHyp</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>E</td>
<td>F</td>
<td>G</td>
<td>H</td>
</tr>
<tr>
<td>J</td>
<td>K</td>
<td>L</td>
<td>M</td>
</tr>
</tbody>
</table>

Figure 2. Scanning electron microscopy (SEM) images of the collagen ultrastructure of heart blood vessels dissected from 20-d-old chicken embryos (1000× magnification). Images A–D: collagen in the inner blood vessel wall; images E–H: collagen between blood vessels; images J–M: collagen in the outer blood vessel wall.
The scanning EM images shown in Figure 3 suggest that the treatments may also have an effect on cartilage, which is primarily composed of Type II collagen. Group C had collagen bundles joined together to form rectangular or circular units. However, the size, thickness and directional orientation of these units were randomly distributed. Group Hyp also had random bundle directional orientation and mixed but mainly circular unit shapes, although the number of observed bundle units was greater than for Group C. The bundle thickness also appeared to be consistent. Groups Ag and AgHyp had larger and thicker

<table>
<thead>
<tr>
<th>Measurement (pixels)</th>
<th>Lattice unit area</th>
<th>Fibre bundle thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental groups</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>102.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyp</td>
<td>115.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ag</td>
<td>116.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AgHyp</td>
<td>589.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>ANOVA (one-way)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM†</td>
<td>93.8</td>
<td>5.18</td>
</tr>
<tr>
<td>p-value</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>ANOVA (two-way)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>93.8</td>
<td>5.18</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyp</td>
<td>0.01</td>
<td>0.08</td>
</tr>
<tr>
<td>Ag</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Notes: †SEM, Standard error of the mean; a,b Means not sharing the same superscript are significantly different (p ≤ 0.05).

Group C Group Hyp Group Ag Group AgHyp

Figure 3. SEM images of the collagen ultrastructure of sternum dissected from 20-d-old chicken embryos (1000× magnification). Diverse pictures of the same group differ between each other in the fragment of the surface from which they were taken.

The scanning EM images shown in Figure 3 suggest that the treatments may also have an effect on cartilage, which is primarily composed of Type II collagen. Group C had collagen bundles joined together to form rectangular or circular units. However, the size, thickness and directional orientation of these units were randomly distributed. Group Hyp also had random bundle directional orientation and mixed but mainly circular unit shapes, although the number of observed bundle units was greater than for Group C. The bundle thickness also appeared to be consistent. Groups Ag and AgHyp had larger and thicker
bundle units than Groups C and Hyp and although their bundle thicknesses varied, there seemed to be a common directional orientation among the thicker bundles. Group AgHyp was observed to have the greatest size and thickness of bundles.

4. Discussion

In this study we set out to confirm whether the in ovo administration of silver nanoparticles supplemented with hydroxyproline can improve the connective tissue growth and development within chicken embryos. FGF-2 and VEGF-A are mitogens associated with angiogenesis by stimulating cell proliferation (Velleman 2002) and their activation is largely dependent on cells that bind heparin sulphate from the extracellular matrix and basement membranes (Larrain et al. 1997). FGF-2 and VEGF-A stimulate more than 20 genes encoding factors that regulate many processes including brain development, limb development and cytoprotection (Baguma-Nibasheka et al. 2007). In this study, the measurements of FGF-2 and VEGF-A mRNA levels suggest that the mitogenesis is increased above the Control group level in all treatment groups. This is because levels of mRNA are directly correlated to gene expression and can indicate the biochemical activity of proteins involved in the remodelling of collagen. However, these measurements lack information about transcriptional regulation and therefore may not indicate the quantity of protein that is produced. For this reason, it was also necessary to determine the levels of protein expression of FGF-2 and VEGF-A.

The amount of FGF-2, VEGF-A and total protein confirmed the experimental observation at mRNA level. The increase in FGF-2 protein was statistically significant for Groups Hyp and AgHyp, while VEGF-A was apparently increased by AgHyp treatment. In addition, the observation that more significant changes are measured for FGF-2 rather than for VEGF-A is understandable as VEGF-A is heavily regulated. Overexpression of VEGF-A can form improper patterning in blood vessels, although this was not observed in our experiments. Altogether, these results suggest that the administration of the AgHyp could cause a significant increase in angiogenesis, which has also been suggested for Ag (Hotowy et al. 2012; Orlowski et al. 2012) and Hyp (Ahanger et al. 2011), administrated separately. The conclusion of our molecular analyses at the mRNA and protein levels is that AgHyp treatment improves connective tissue growth and development via the activation of gene expression. The results of these activating events could be seen in changes of collagen structure, observed after embryos had fully developed (day 20) according to Hamburger and Hamilton (1951) and provide an overall view of the treatment effects during embryogenesis. However, to check whether the AgHyp treatment has prolonged effects on collagen structure in fully grown birds, more experiments need to be performed.

The observed significant increases in lattice area and in bundle thickness of collagen fibre in Group AgHyp compared to the other groups could be an indicator of improved tensile strength. The mechanical properties of this tissue are determined by the mixture of fibril types, crosslinks and the packing density of parallel fibres within large fibre bundles (Chvapil 1967; Gay and Miller 1978). Fibrils can be oriented differently according to tissue type and fibrils can align parallel to each other to form fibres or bundles. The more fibrils that are present per bundle, the less likely it is that the breakage of any individual fibril under stress will affect the mechanics of the whole bundle. Furthermore, if the individual fibrils have greater cross-sections, each of them can tolerate more shear stress (Ottani et al. 2001) and
Therefore, the greater lattice unit area of collagen fibre may be a good indicator of improved elasticity. While fibre separation may just suggest higher levels of tissue hydration, the ability of structures to locally compress, contort and absorb energy is important. This is especially true in vascular tissue, which undergoes rapid cyclic stretch (Imamura et al. 2007). Other studies of vascular collagen suggest that this is indicative of the collagen subtypes within the fibres. Type I collagen provides traction firmness, while Type III collagen enhances dilatability and elasticity (Haviarova et al. 2008). In the present experiment the collagen subtype composition was not determined; however, other studies have suggested that higher cell density causes fibroblasts to produce relatively more Type III than Type I collagen (Abe et al. 1979).

The following scenario is a possible model of how AgHyp could interact with collagen, although the exact mechanism is still unclear. Initially, AgHyp self-assembles into a biocomplex in vitro and once injected into the embryo, the AgHyp complex eventually enters the bloodstream. During transport, the structure of the biocomplex may protect it from being targeted for degradation. If so, the AgHyp could be preferentially accumulated in the extracellular matrix for future use during angiogenesis. This mirrors the distribution process for proline. During angiogenesis, collagen alpha chains self-assemble in the rough endoplasmic reticulum with some chains incorporating Hyp instead of proline. A higher Hyp content creates a more stable structure and in addition, hydroxylation of proline avoids regulation of collagen prolyl 4-hydroxylase. This results in more high-Hyp content alpha chains (than normal chains) reaching the Golgi apparatus for secretion into the extracellular space. Outside the cell, alpha chains self-assemble into collagen. While it is not clear if the AgHyp biomolecule or just Hyp is directly incorporated into the alpha chain, it is likely that Ag nanoparticles are also transported to the collagen self-assembly site in some form. Electron microscope research (Vidal and Joazeiro 2002) shows that silver nanoparticles readily bind to collagen fibres in a highly ordered manner and contribute to bonds between fibres (Ionita et al. 2010). Other studies (Kwan et al. 2011) show that silver nanoparticles cause increased deposition of collagen and build aligned collagen formations that have greater tensile strength than normal collagen.

5. Conclusions

The experimental solutions administered in ovo to chicken embryos did not harm embryos. However, the treatments with Hyp, Ag and AgHyp generally upregulated mRNA and protein levels of FGF-2 and VEGF-A. Furthermore, Hyp, Ag and, in particular, a complex of AgHyp increased lattice size and bundle thickness of collagen fibre in blood vessels. This may indicate an improvement in blood vessel elasticity and tensile strength. The general conclusion from this study is that AgHyp treatment may help to build a stronger and longer lasting form of collagen fibres.

Funding

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