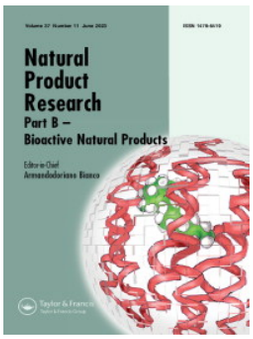

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Steady state plasma and tissue distribution of low molecular weight hyaluronic acid after oral administration in mice

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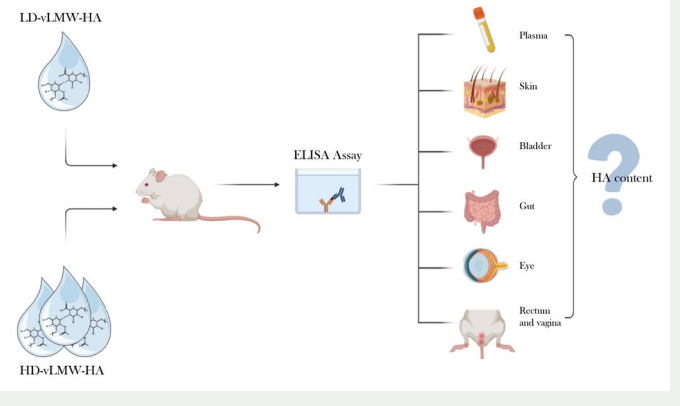
ABSTRACT

The oral administration is probably the most used and largely applicable method, even if absorption across the intestinal epithelium is a limiting factor that can invalidate the achievement of a therapy. The aim of this study was to assess the steady state bioavailability of very low molecular weight hyaluronic acid (vLMW-HA) and its distribution in different districts of mice. Adult female C57BL6/J mice ($n = 26$) were divided in three groups and orally treated for 7 days with: saline solution (SHAM-HA), high dose of vLMW-HA (5 kDa; 500 mg/kg/day; HD-vLMW-HA), and low dose of vLMW-HA (5 kDa; 100 mg/kg/day; LD-vLMW-HA). HA content was quantified in plasma, skin, bladder, gut, rectum, vagina, and eyes with ELISA assay at the end of treatment. HA level significantly increased after treatment with HD-vLMW-HA in all analyzed tissues and plasma. Therefore, vLMW-HA easy absorption and distribution after the oral intake opens new possibilities for future biomedical applications.



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1. Introduction

The nature of drugs or supplements along with the absorption efficacy are fundamental components for a successful therapy. Among the several delivery routes commonly used for the administration of drugs or supplements, the oral one is probably the most used and represents a widely applicable method for the ease of administration and the high patient compliance (Homayun et al. 2019). Nevertheless, when oral drugs are ingested, they encounter different challenges before reaching their target (Leal et al. 2017) and may have some limitations due to the low bioavailability or short half-life. Hyaluronic acid (HA) is one of the most interesting, versatile, and useful natural macromolecules. Despite its simple chemical structure, HA plays a key role in several biological processes and in different cell types and tissues, both in normal and pathological conditions (Fraser et al. 1997). In the last few years this molecule has gained great attention and found an extraordinarily broad range of biomedical applications (Vasvani et al. 2020; Valachová and Šoltés 2021). The most common administration route of HA is intra-articular injection for the treatment of osteoarthritis (OA) and rheumatoid arthritis (Battaglia et al. 2013), or filler injection in the field of aesthetic medicine (Lipko-Godlewska et al. 2021). Over the last years, also oral administration of exogenous HA has gained attention as supplementary therapy to treat local diseases, as atrophic vaginitis, or relieving chronic pain (La Galia et al. 2014; Jensen et al. 2015; Göllner et al. 2017). In this regard, the molecular weight of hyaluronic acid is crucial for the functions exerted and can determine its fate once ingested (Kimura et al. 2016) given that several factors may influence the gastrointestinal absorption. Not many reports directly measured HA absorption. Some studies examined HA in the gastrointestinal tract and its translocation to thyroid glands, stomach, kidney, and bladder using radioisotopes (Balogh et al. 2008), particularly focusing on the high molecular weight form (HMW-HA >500kDa). Nevertheless, despite these few studies, the distribution of HA is still unclear, especially for the low molecular weight form (LMW-HA <500 kDa). LMWHA when locally applied, can be easier absorbed by epithelia compared with HMWHA (Essendoubi et al. 2016). This property can be useful to alleviate, for example, all the symptoms associated with vaginal atrophy, as severe grade of dryness, itching, burning and dyspareunia (Costantino and Guaraldi 2008). Moreover, by interacting with several cells involved in proliferation processes (Jiang et al. 2011) and the immune system, LMW-HA can contribute to restore the physiological architecture of the damaged mucosa (Nakamura et al. 2004; Chang et al. 2007; Kavasi et al. 2017). Despite local application has been widely investigated, oral administration lacks evidence.

Therefore, the aim of the study was to assess the bioavailability and distribution of the very low molecular weight hyaluronic acid (vLMW-HA <10kDa) in various districts of mice, after oral supplementation.

2. Results and discussion

Quantikine™ Hyaluronic acid Immunoassay were performed to evaluate the HA levels in the plasma, skin, bladder, gut, rectum, vagina, and eye after oral treatment of mice with HD-vLMW-HA and LD-vLMW-HA. In the plasma, the mean value of HA was

420,3 ng/ml. After 7 days of treatment with the lower dose of vLMW-HA (100 mg/kg), the steady state HA in plasma did not significantly change compared to SHAM-HA group; instead, the higher dose of vLMW-HA (500 mg/kg) induced a significant increase of HA level compared to SHAM-HA group and LD-vLMW-HA-treated group (682,7 ng/ml) (Figure 1a).

Regarding tissue distribution samples analyzed at the end of the study demonstrated a similar pattern of content: the mean level of HA in the skin was 4584 ng/ml. The dose of 100 mg/kg did not significantly change HA content (5138 ng/ml), while the 500 mg/kg dose caused a significant increase (6600 ng/ml) ($p < 0.01$, Figure 1b).

In bladder samples, the mean level of HA was 2161 ng/ml. Both LD-vLMW-HA (2626 ng/ml, $p < 0,05$) and HD-vLMW-HA (3323 ng/ml $p < 0,01$) treatments markedly increased HA levels compared to the SHAM-HA group (Figure 2a), probably due to the accumulation of hyaluronic fragments during excretion. On the other hand, HA mean level in gut samples were 4470 ng/ml: they significantly increased only in the HD-vLMW-HA-treated group (6901 ng/ml $p < 0,01$) compared to the LD-vLMW-HA-treated group (5272 ng/ml), and the SHAM-HA group (Figure 2b).

HA levels were also evaluated in rectal mucosa, vagina, and eye samples. The mean values of HA were 2993 ng/ml, 2674 ng/ml, and 4020 ng/ml respectively. LD-vLMW-HA administration did not modify HA levels compared to SHAM-HA group whereas, mice treated with HD-vLMW-HA showed a significantly increase in HA levels (4692 ng/ml,

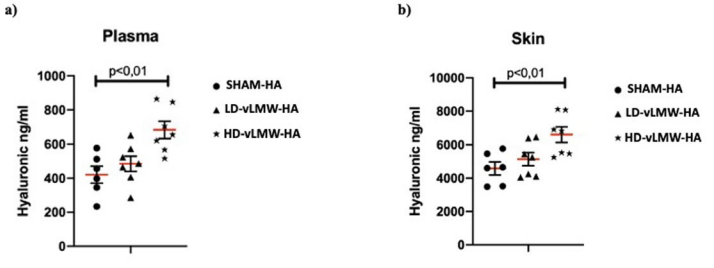

Figure 1. HA levels evaluated by Quantikine™ Hyaluronic acid Immunoassay in plasma (a) and skin (b) samples from SHAM-HA group, LD-vLMW-HA treated group and HD-vLMW-HA treated group.

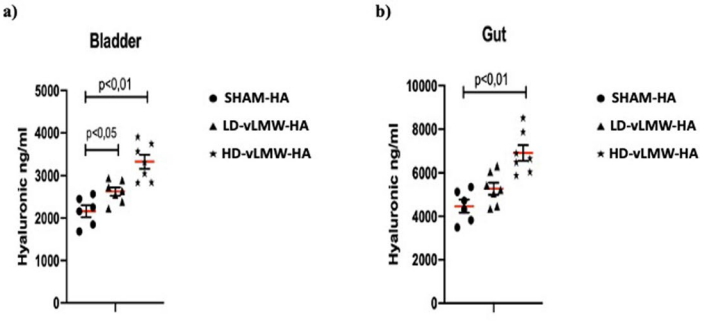

Figure 2. HA levels evaluated by Quantikine™ Hyaluronic acid Immunoassay in bladder (a) and gut (b) samples from SHAM-HA group, LD-vLMW-HA treated group and HD-vLMW-HA treated group.

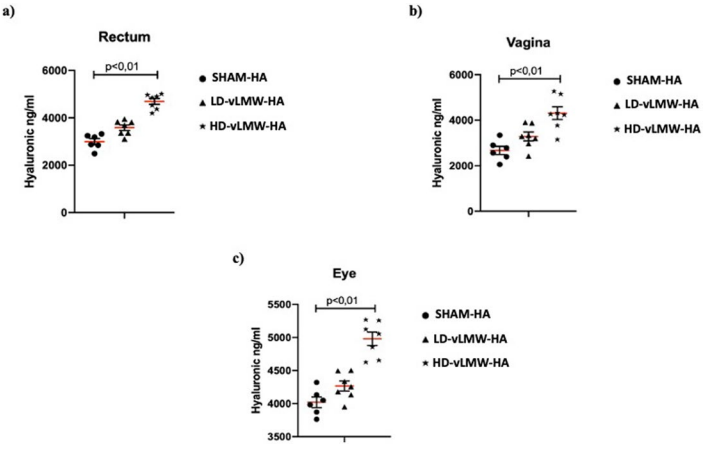

Figure 3. HA levels evaluated by Quantikine™ Hyaluronic acid Immunoassay in rectum (a), vagina (b) and eye (c) samples from SHAM-HA group, LD-vLMW-HA treated group and HD-vLMW-HA treated group.

4314 ng/ml, and 4979 ng/ml respectively; $p < 0{,}01$) compared to the SHAM-HA group and the LD-vLMW-HA-treated group (Figure 3a–c) in all evaluated samples.

This study investigated distribution of vLMW-HA in plasma and different tissues of adult female mice, after its oral uptake. Our results highlighted that hyaluronic acid with a very low molecular weight (5kDA) exhibits a good biodistribution. Indeed, HA level increased in all the analyzed tissues, especially when animals were treated with the highest dose of vLMW-HA (500 mg/kg). In the first part of this study, we quantified HA level in plasma at the steady state after oral administration. In the SHAM-HA group, an average of 420.3 ng/ml of HA was detected. A significant increase of HA level was observed in the HD-vLMW-HA-treated group (682.7 ng/mL, $p < 0.01$) with respect to LD-vLMW-HA-treated group (484.6 ng/ml). In all the analyzed districts (skin, bladder, gut, rectum, vagina, eyes), the higher dose of low molecular weight HA ensured a better absorption with respect to the lower dose.

Different drugs' delivery routes are provided for different purposes and each of them encounters specific barriers before reaching their target. The oral route is probably the simplest way to delivery drugs or supplements given the ease of administration and the high patient compliance. Interestingly, the most challenging step in the oral uptake of a substance is its absorption across the intestinal epithelium. The absorptive properties of HA are still unclear, despite few experiments tested the high molecular weight HA absorption in the gastrointestinal tract and its translocation to tissues by using radiolabeled molecules (Balogh et al. 2008). However, information about the distribution in the various tissues of HA with different molecular weights is still very limited, and results from different studies are sometimes controversial, due to the nature of radioisotopes (Laurent et al. 1991; Komatsu et al. 1999; Kimura et al. 2016). Our study overcomes this limit because no radioisotope was used, instead HA level was directly quantified by using an enzyme-linked immunosorbent assay (ELISA).

Hisada investigated the intestinal permeability of hyaluronic acid with low molecular weight in a CaCo2 cell line model and demonstrated that HA permeability was dose dependent and increased with the reduction of molecular size (Hisada et al. 2008). This is consistent with our results, which highlighted a similar dose dependent absorption rate observable both with low and high dose of vLMW-HA in all the analyzed tissues. Hence, a significant increase in HA content was observed only in the HD-vLMW-HA-treated mice.

Hyaluronic acid is one of the most interesting, peculiar, and useful macromolecules with an important role in almost all areas of biology. Currently, HA is used in several branches of medicine (pulmonology, orthopedics, aesthetic medicine, gynecology, ophthalmology, etc.) and no contraindications or adverse interactions with other drugs have been documented (Bray 2001; Nolan et al. 2006; Ciofalo et al. 2017; Huynh and Priefer 2020). Hyaluronic acid can modulate several pathways and its modulatory function not only depends on the balance between its synthesis and degradation, but it is mainly due to its molecular weight which determines hyaluronic acid activities. In some case high molecular (HMW) and very low/low molecular weight (LMW) hyaluronic acid can exert opposite effects (Cyphert et al. 2015). HMW-HA is a pivotal component of skin integrity and thanks to its hydrophilic nature can increase moisture retention and smoothness of the skin by incorporating water molecules (Sato et al. 2002). Thanks to its water retention properties and viscoelasticity, HA favors the healing of corneal and conjunctival epithelium (Aragona et al. 2002; Gomes et al. 2004), determining a reduction of ocular surface inflammation for the treatment of signs and symptoms of moderate to severe dry eye syndrome. Furthermore, HMW-HA exhibits anti-inflammatory and antiangiogenic properties, promotes epithelial cell integrity and, thanks to the high viscosity, it is a suitable lubricant agent in the synovial joint fluid favoring the protection of the articular cartilage (Tamer 2013). Moreover, HMW-HA has beneficial roles in wound healing repair, and immunosuppression, thus controlling the levels of inflammatory cytokines and the migration of stem cells (Jiang et al. 2011). On the other hand, LMW-HA, during some environmental and pathological conditions including asthma, pulmonary fibrosis, and hypertension, exhibits pro-inflammatory and pro-angiogenic properties, by promoting extracellular matrix (ECM) remodeling (Heldin et al. 2019). Experimental studies demonstrated that treatment with vLMW-HA could be appropriate for different applications. For example, vLMW-HA exerts a reparatory action in dermal excisional wounds (Gao et al. 2008). In sites of inflammation or tissue injury, it induces activation of keratinocytes and improves the release of β-defensins, without an inflammatory response, thus ameliorating the self-defense of the skin for the protection of cutaneous tissue from infection by microorganisms (Gariboldi et al. 2008). Oral administration of vLMW-HA ameliorates vaginal atrophy, improves epithelium thickness, and increases the number of epithelial layers in postmenopausal women (La Galia et al. 2014). Moreover, vLMW-HA could also be used as preventive treatment in pathological conditions such as patients who undergo radiotherapy (RT) for prostate cancers (Piro and Marafioti 2018). Often, urinary toxicity is an unpleasant side effect of postoperative radiotherapy, in a pilot study, treatment with oral vLMW-HA completely reduced toxicity in 89% of patients affected by prostate cancer and undergoing RT (Piro and Marafioti 2018).

3. Conclusion

Hyaluronic acid is a key molecule that can cover a wide range of clinical applications, thanks to its different molecular weights. The success of a therapy often depends on how a supplement is distributed after its absorption. Oral delivery route is often chosen, because of the ease of administration and the high patient compliance; nevertheless, many challenges are encountered before supplement reaches its target. So, it is necessary to increase knowledge of the exact mechanism by which oral hyaluronic acid is distributed when orally administrated. Several pieces of evidence exist about the 'fate' of HMW-HA, while lacks exist regarding the very low molecular weight. Given its properties and its usefulness in many branches of medicine, we analyzed its level and distribution in plasma and other tissues, to open new possibilities for future biomedical and clinical perspectives.

Author contributions

Conceptualization, F.M.; supervision A.B.; writing—original draft, F.M.; writing—review and editing, A.B.; methodology, G.P.; validation, N.I. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

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