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ORIGINAL ARTICLE

Hyaluronic acid: a unique topical vehicle for the localized delivery of drugs to the skin

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ABSTRACT

Hyaluronic acid (HA) is a naturally occurring polyanionic, polysaccharide that consists of *N*-acetyl-D-glucosamine and β-glucoronic acid. It is present in the intercellular matrix of most vertebrate connective tissues especially skin where it has a protective, structure stabilizing and shock-absorbing role. The unique viscoelastic nature of HA along with its biocompatibility and non-immunogenicity has led to its use in a number of clinical applications, which include: the supplementation of joint fluid in arthritis; as a surgical aid in eye surgery; and to facilitate the healing and regeneration of surgical wounds. More recently, HA has been investigated as a drug delivery agent for various routes of administration, including ophthalmic, nasal, pulmonary, parenteral and topical. In fact, regulatory approval in the USA, Canada and Europe was granted recently for 3% diclofenac in 2.5% HA gel, Solaraze®, for the topical treatment of actinic keratoses, which is the third most common skin complaint in the USA. The gel is well tolerated, safe and efficacious and provides an attractive, cost-effective alternative to cryoablation, curettage or dermabrasion, or treatment with 5-fluorouracil. The purpose of this review is to describe briefly the physical, chemical and biological properties of HA together with some details of its medical and pharmaceutical uses with emphasis on this more recent topical application.

Key words: actinic keratoses, dermatology, diclofenac, hyaluronic acid, Solaraze, topical drug delivery

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Introduction

Hyaluronic acid (HA) was discovered in bovine vitreous humour by Meyer and Palmer in 1934.[1] It is most frequently referred to as HA due to the fact that it exists *in vivo* as a polyanion and not in the protonated acid form. HA is ubiquitous in that it is distributed widely in vertebrates and present as a component of the cell coat of many strains of bacteria.[2] Commercially produced HA is isolated either from animal sources, within the synovial fluid, umbilical cord, skin, and rooster comb, or from bacteria through a process of fermentation or direct isolation. The molecular weight of HA is heavily dependent on its source; however, refinement of these isolation processes has resulted in the commercial availability of numerous molecular weight grades extending up to a maximum of 5000 kDa.[3] Extensive studies on the chemical and physicochemical properties of HA and its physiological role in humans, together with its versatile properties, such as its biocompatibility, non-immunogenicity, biodegradability and viscoelasticity, have proved that it is an ideal biomaterial for cosmetic, medical and pharmaceutical applications. Several of HA's important physicochemical properties are molecular weight dependent and therefore discrete differences in function over the wide range of commercially available molecular weights enables HA to be used in a diverse set of applications. In addition, chemical modification of HA can produce a more mechanically and chemically robust material that still retains its biocompatibility and biodegradability. Methods detailing the synthetic transformation of HA and the resulting applications in cosmetic, medical and pharmaceutical applications have been reviewed extensively previously[4–11] and are not included in this paper. The

purpose of this review is to describe briefly the physical, chemical and biological properties of HA together with details of its medical and pharmaceutical uses with emphasis on the more recent topical applications.

Physical, chemical and biological properties of hyaluronic acid

The precise chemical structure of HA, which contains repeating units of D-glucoronic acid and *N*-acetyl-D-glucosamine, was first determined by Weissman and Meyer in 1954.[2] The primary structure of the polysaccharide comprises of an unbranched linear chain with the monosaccharides linked together through alternating $\beta_{1,3}$ and $\beta_{1,4}$ glycosidic bonds (fig. 1a).[2,12,13] Hydrophobic faces exist within the secondary structure of HA, formed by the axial hydrogen atoms of about eight CH groups on the alternating sides of the molecule. Such hydrophobic patches, energetically favour the formation of meshwork-like β-sheet tertiary structure as a result of molecular aggregation (fig. 1b). The tertiary structure is stabilized by the presence of intermolecular hydrogen bonding.[13] The hydrophobic and hydrogen bonding interactions in combination with the countering electrostatic repulsion, enable large numbers of molecules to aggregate leading to the formation of molecular networks (matrices) of HA (fig. 1c).

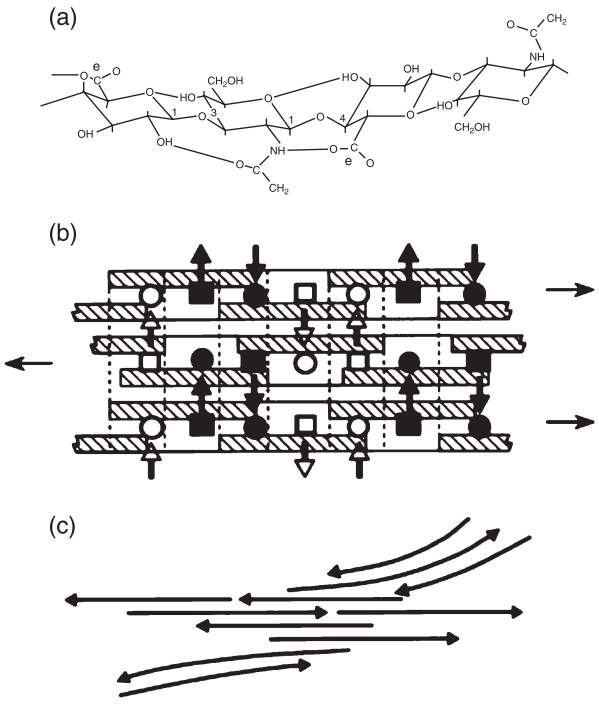

fig. 1 The primary, secondary and tertiary structures of hyaluronic acid (HA) in solutions. (a) The primary structure of HA consists of repeating disaccharide units of D-glucoronic acid and *N*-acetyl-D-glucosamine with up to five hydrogen bonds existing between each two neighbouring disaccharides, while the secondary structures are formed as tape-like twofold helices by twisting each disaccharide unit through 180° compared with those ahead and behind it in the chain. (b) The β-sheet tertiary structure tertiary structure is energetically stabilized by interactions between hydrophobic patches (hatched) and intermolecular hydrogen bonding between the acetamido (■ and □) and carboxylate groups (● and ○). (c) Schematic networks of HA molecules as a results of inter-molecular aggregation (Reprinted with permission from Scott and Heatley[13]).

HA in aqueous solution has been reported to undergo a transition from Newtonian to non-Newtonian characteristics with increasing molecular weight, concentration or shear rate.[14] In addition, the higher the molecular weight and concentration of HA, the higher the viscoelasticity the solutions possess.[14–18] The viscoelasticity of HA in aqueous solution is pH dependent and effected by the ionic strength of its environment.[17,19–21] HA has a pKa value of about 3.0 and therefore, a change in pH will effect the extent of ionization of the HA chains. A shift in ionization alters the intermolecular interactions between the HA molecules, which changes the rheological properties of the compound.

HA in combination with other glycosaminoglycans, such as dermatan sulphate,[22] chondroitin sulphate and keratin sulphate[23] are prominent in tissues such as the skin. HA is found to exist together with protein cores (aggrecans) to which others such as chondroitin sulphate, keratin sulphate and dermatan sulphate are attached. As its name implies, aggrecan is composed of very large proteoglycan aggregates. The most important property of these molecules is their ability to bind to water and this induces the proteoglycans to become hydrated to such an extent that a gel-like system is formed. The networks of proteoglycan–HA aggregates shift the Newtonian region to lower shear rates and these gels have an increased dynamic viscoelasticity relative to HA–HA networks.

HA is found in almost all vertebrate organs, but most abundantly in the extracellular matrix of soft connective tissues. HA is particularly abundant in mammalian skin where it constitutes a high fraction of the extracellular matrix of the dermis.[24,25] The estimated total amount of HA in human skin has been reported to be 5 g,[26] about a third of the total amount of HA believed to be present within the entire human body.[27] HA is present in both the dermis (≈ 0.5 mg/g wet tissue) and the epidermis (≈ 0.1 mg/g wet tissue). Interestingly, while the dermis consists primarily of extracellular matrix with a sparse population of cells, the epidermis is the reverse; the keratinocytes fill all but a few per cent of the tissue. Thus, the actual concentration of HA in the matrix around the cells in the epidermis (estimated to be 2–4 mg/mL) is an order of magnitude higher than in the dermis (estimated to be ≈ 0.5 mg/mL) and approximately the same as that in the umbilical cord. In addition, Sakai *et al.*[28] have shown that HA is derived from keratinocytes beneath the stratum corneum, and is present in the normal stratum corneum.

Many physiological functions of HA are thought to relate to its molecular characteristics, including its physiochemical

Table 1 Summary of the medical applications of hyaluronic acid

Disease state	Applications	Commercial products	Publications
Osteoarthritis	Lubrication and mechanical support for the joints	Hyalgan® (Fidia, Italy), Artz® (Seikagaku, Japan) ORTHOVISC® (Anika, USA) Healon®, Opegan® and Opelead®	Hochburg, 2000,[39] Altman, 2000,[40] Dougados, 2000,[41] Guidolin *et al.*, 2001,[42] Maheu *et al.*, 2002,[43] Barrett and Siviero, 2002,[44] Miltner *et al.*, 2002,[45] Tascioglu and Oner, 2003,[46] Uthman *et al.*, 2003,[47] Kelly *et al.*, 2003,[48] Hamburger *et al.*, 2003,[49] Kirwan, 2001,[50] Ghosh and Guidolin, 2002,[51] Mabuchi *et al.*, 1999,[158] Balazs, 2003,[52] Fraser *et al.*, 1993,[53] Zhu and Granick, 2003.[54]
Surgery and wound healing	Implantation of artificial intraocular lens Viscoelastic gel	Bionect®, Connettivina® and Jossalind®	Ghosh and Jassal, 2002,[55] Risbert, 1997,[56] Inoue and Katakami, 1993,[57] Miyazaki *et al.*, 1996,[58] Stiebel-Kalish *et al.*, 1998,[59] Tani *et al.*, 2002,[60] Vazquez *et al.*, 2003,[61] Soldati *et al.*, 1999,[62] Ortonne, 1996,[63] Cantor *et al.*, 1998,[64] Turino and Cantor, 2003.[65]
Embryo implantation	Culture media for the use of *invitro* fertilization	EmbryoGlue® (Vitrolife, USA)	Simon *et al.*, 2003,[66] Gardner *et al.*, 1999,[67] Vanos *et al.*, 1991,[68] Kemmann, 1998,[69] Suchanek *et al.*, 1994,[70] Joly *et al.*, 1992,[71] Gardner, 2003,[72] Lane *et al.*, 2003,[73] Figueiredo *et al.*, 2002,[74] Miyano *et al.*, 1994,[75] Kano *et al.*, 1998,[76] Abeydeera, 2002,[77] Jaakma *et al.*, 1997,[78] Furnus *et al.*, 1998,[79] Jang *et al.*, 2003.[80]

properties, its specific interactions with hyaladhereins, such as CD44 and RHAMM (receptor for HA-mediated motility), and its mediating effect on cell signalling and behaviour.[29] Recently, understanding of the role of HA in the mediation of physiological functions via interaction with binding proteins and cell surface receptors, e.g. CD44[30–33] in the epidermis has emerged suggesting that the molecule may be involved in cell function.[34] However, the exact mechanisms by which different signals are activated have not yet been completely defined.[35,36]

Medical and drug delivery applications of hyaluronic acid

HA's viscoelastic matrix can act as a strong biocompatible support material and therefore is commonly used as growth scaffold in surgery, wound healing and embryology. In addition, administration of purified high molecular weight HA into orthopaedic joints can restore the desirable rheological properties and alleviate some of the symptoms of osteoarthritis.[37,38] The success of the medical applications of HA has led to the production of several successful commercial products, which have been extensively reviewed previously.[39–80] Table 1 summarizes both the medical applications and the commonly used commercial preparations containing HA used within this field.

HA has also been extensively studied in ophthalmic,[81–95] nasal[96,97] and parenteral drug delivery.[98–107] In addition, more novel applications including, pulmonary,[108,109] implantation[110,111] and gene delivery[112,113] have also been suggested. Generally, HA is thought to act as either a mucoadhesive and retain the drug at its site of action/absorption or to modify the *in vivo* release/absorption rate of the therapeutic agent. A summary of the drug delivery applications of HA is shown in Table 2.

Dermatological applications

Cosmetic uses of hyaluronic acid

HA has been extensively utilized in cosmetic products because of its viscoelastic properties and excellent biocompatibility.[114] Application of HA containing cosmetic products to the skin is reported to moisturize and restore elasticity thereby achieving an antiwrinkle effect, albeit no rigorous scientific proof exists to substantiate this claim. HA-based cosmetic formulations or sunscreens may also be capable of protecting the skin against ultraviolet irradiation due to the free radical scavenging properties of HA.[115]

HA, either in a stabilized form or in combination with other polymers, is used as a component of commercial dermal fillers (e.g. Hylaform®, Restylane® and Dermalive®) in cosmetic surgery. It is reported that injection of such products into the dermis, can reduce facial lines and wrinkles in the long term with fewer side-effects and better tolerability compared with the use of collagen.[116–118] The main side-effect may be an allergic reaction, possibly due to impurities present in HA.[119,120] Lin *et al.*[121] have also investigated the feasibility of using HA as an alternative implant filler material to silicone gel in plastic surgery. These workers found that when using HA, the implanted organ structure was visually better than that obtained using silicone gel and saline implants, Moreover there were no reported *in vivo* side-effects 1 year after the implantation.

Table 2 Summary of the drug delivery applications of hyaluronic acid

Route	Justification	Therapeutic agents	Publications
Ophthalmic	Increased ocular residence of drug, which can lead to increased bioavailability	Pilocarpine, tropicamide, timolol, gentimycin, tobramycin, arecaidine polyester, (S) aceclidine	Jarvinen *et al.*, 1995,[81] Sasaki *et al.*, 1996,[82] Gurny *et al.*, 1987,[83] Camber *et al.*, 1987,[84] Camber and Edman, 1989,[85] Saettone *et al.*, 1994,[86] Saettone *et al.*, 1991,[87] Bucolo *et al.*, 1998,[88] Bucolo and Mangiafico, 1999,[89] Herrero-Vanrell *et al.*, 2000,[90] Moreira *et al.*, 1991,[91,92] Bernatchez *et al.*, 1993,[93] Gandolfi *et al.*, 1992,[94] Langer *et al.*, 1997.[95]
Nasal	Bioadhesion resulting in increased bioavailability	Xylometazoline, vasopressin, gentamycin	Morimoto *et al.*, 1991,[96] Lim *et al.*, 2002.[97]
Pulmonary	Absorption enhancer and dissolution rate modification	Insulin	Morimoto *et al.*, 2001,[108] Surendrakumar *et al.*, 2003.[109]
Parenteral	Drug carrier and facilitator of liposomal entrapment	Taxol, superoxide dismutase, human recombinant insulin-like growth factor, doxorubicin	Drobnik, 1991,[98] Sakurai *et al.*, 1997,[99] Luo and Prestwich, 1999,[100] Luo *et al.*, 2000[101] Prisell *et al.*, 1992,[102] Yerushalmi *et al.*, 1994,[103] Yerushalmi and Margalit, 1998,[104] Peer and Margalit, 2000,[105] Eliaz and Szoka, 2001,[106] Peer *et al.*, 2003.[107]
Implant	Dissolution rate modification	Insulin	Surini *et al.*, 2003,[110] Takayama *et al.*, 1990.[111]
Gene	Dissolution rate modification and protection	Plasmid DNA/monoclonal antibodies	Yun *et al.*, 2004,[112] Kim *et al.*, 2003.[113]

Hyaluronic acid in topical drug delivery

Background

Topical delivery for the treatment of skin disorders offers numerous potential advantages over systemic therapies, such as those involving the use of oral or parenteral products. These include avoidance of hepatic first-pass metabolism, improved patient compliance and ease of access to the absorbing membrane, i.e. the skin. In addition, by directly administering the drug to the pathological site, any adverse effects associated with systemic toxicity can be minimized. However, the targeted delivery of drugs for the treatment of topical disorders is not trivial.

The physiological function of the stratum corneum, the outermost and non-viable layer of the skin, is to act as a protective barrier for the body and as such it is particularly effective at preventing the permeation of hydrophilic molecules including some drugs into deeper skin layers, where viable cells are located. Thus, additional methods of chemical or physical penetration enhancement are often required to traverse this barrier. However, the use of such methods also promotes the transport of drug all the way through the skin into the blood stream. This can be a prime objective if systemic effects are required (as with transdermal patches), but if the target receptor for the drug lies within the epidermis, it represents the potential cause of side-effects. Consequently, in topically applied formulations there is a need for a system that helps the drug to negotiate successfully the stratum corneum, but then avoids further penetration all the way across the deeper layers of the skin (the dermis) and into the blood stream. Obviously, this requires a finely balanced system and is not easy to achieve. Relatively few investigations have been conducted into the development of such delivery systems for drug targeting to the skin, although one such system that is receiving increasing attention is HA,[122] which has been shown to localize delivery of drug to the epidermis when applied topically, as described below.

Three per cent diclofenac in 2.5% hyaluronic acid gel, Solaraze®

Recently a formulation, marketed as Solaraze, was approved in the USA, Canada and most European countries for the topical treatment of actinic keratoses (AK) the third most common skin complaint in the US.[123–126] AK are relatively common skin lesions that result from excessive sun exposure. They are most common on the scalp, face and/or dorsum of the hands in light-skinned persons aged ≥ 50 years, particularly those with a history of occupational exposure to the sun. AK are now considered to be synonymous with squamous cell carcinoma (SCC) *in situ* with growing evidence that AK and SCC lie on a clinical, histological, cytological and molecular continuum.[127] Although AK frequently do progress to invasive SCC it is not currently possible to predict which lesions will progress. Estimates of the rate of progression range from 0.025 to 16% per year.[128] The rationale for treating AK is thus the elimination of incipient malignant cells during a phase in which they are curable rather than waiting for progression to a more advanced phase with a less favourable prognosis or treatment-associated morbidity. In Australia, where AK and skin cancer are more common than anywhere else in the world, the prevalence was estimated to be 40–50% in people 40 years of age and older, which equates to a minimum prevalence rate of 15 000/100 000 per year.[129] In a study carried out in New South Wales, involving 1034 subjects aged 60 years and over, the AK prevalence rate was 23% (95% CI 19.5–26.5).[130]

Table 3 A comparison of complete clearance of actinic keratoses lesions by location using topical 3% diclofenac in 2.5% hyaluronic acid gel compared with the vehicle alone at three treatment durations. Summary of Del Rosso[125], Rivers and Mclean[131] and Wolf *et al.*[134]

Body location	Scalp	Forehead	Face	Arm/forearm	Back of hand
Diclofenac	8/22 (36%)	43/109 (39%)	26/55 (47%)	9/21 (43%)	9/50 (18%)
Vehicle	3/24 (13%)	21/111 (19%)	11/56 (20%)	4/20 (20%)	5/48 (10%)
P-value	0.0903	0.0013	0.0016	0.2043	0.3662

Extensive randomized, double blind, 3% diclofenac in 2.5% HA gel vehicle-controlled clinical studies,[131–134] as reviewed by Jarvis and Figgitt,[133] have been performed and have shown that 3% diclofenac in 2.5% HA gel is well tolerated, safe and efficacious. The most common adverse events associated with the use of the gel were at the application site and included contact dermatitis, dry skin, rash and exfoliation. There were no serious adverse events or deaths that were considered to be treatment related. Withdrawals due to adverse events were found very occasionally and were primarily due to skin irritation.

The percentage of patients having complete clearance of AK lesions at 30 days follow-up in the three phase III studies for all body locations and specific sites are detailed in Table 3 and Table 4, respectively. Three per cent diclofenac in 2.5% HA gel appears to be most effective on lesions located on the face and forehead and is also clear that the efficacy of the gel increases in proportion to the duration of treatment in patients (see Table 4). Thus sufficient gel should be applied to lesion areas twice daily for a duration of 60–90 days. The formulation does appear to provide an attractive, cost-effective alternative to cryoablation, curettage or dermabrasion, or treatment with 5-fluorouracil, which can have considerable adverse side-effects.

Hyaluronic acid's topical mechanism of action

Although the exact mechanism of action of diclofenac in the Solaraze® formulation remains unclear, it has long been established that some non-steroidal anti-inflammatory drugs have an antitumorigenic effect,[135–137] which may be attributable to non-steroidal anti-inflammatory drug-mediated inhibition of the cyclooxygenase pathway and specifically to reduced prostaglandin E_2 synthesis. Prostaglandin E_2 is known to attenuate cell-mediated immunity; in particular this prostaglandin inhibits natural killer cell cytotoxicity,[138] mitogen-induced lymphocyte proliferation[139] and macrophage proliferation and antitumour cytotoxicity.[140]

The beneficial effect of HA on the topical delivery of diclofenac has also been rigorously investigated *in vitro*.[122,134,141–148] Brown *et al.*[141–143] have shown that HA enhances significantly the partitioning of diclofenac into human skin and its retention and localization in the epidermis when compared with an aqueous control, other glycosaminoglycans (e.g. chondroitin sulphate) and commonly used pharmaceutically acceptable gelling agents (e.g. sodium carboxymethyl cellulose) at either molar or rheologically equivalent concentrations (see fig. 2). Such results

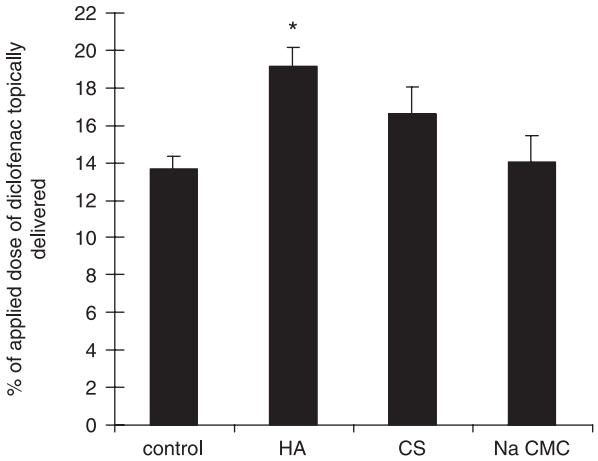

fig. 2 The effect of 1% w/w polysaccharide on the percentage of applied diclofenac (1.75% w/w) delivered to the skin, 48 h after application of the formulation ($n = 4$, mean ± SD, $^*P < 0.001$ compared with control).

Table 4 A comparison of complete clearance of actinic keratoses lesions at all body locations using a topical 3% diclofenac in 2.5% hyaluronic acid (HA) gel compared with the vehicle alone at three treatment durations

Treatment duration (days)	Diclofenac 3% HA 2.5% gel	Vehicle	*P*-value	Clinical studies
90	27/58 (47%)	11/59 (19%)	< 0.001	Wolf *et al.*[134]
60	15/48 (31%)	5/49 (10%)	0.021	Rivers and Mclean[131]
30	7/49 (14%)	2/49 (4%)	0.221	Rivers and Mclean [131]

have been confirmed in further studies where HA was shown to minimize the percutaneous absorption of diclofenac[122,134,144] (fig. 3) indicating the formation of a depot or reservoir of drug in the epidermis, which was confirmed using autoradiography.[145] In addition, Lin and Maibach[148] demonstrated that HA delivered twice as much diclofenac to the epidermis over 24 h when compared with an aqueous control and sodium carboxymethyl cellulose. HA has also been found to produce similar effects with ibuprofen,[141,143] clindamycin phosphate[149] and ciclosporin.[146,147]

The *in vitro* data have been supported by preclinical studies where radiolabelled HA was found not only to penetrate the skin of nude mice and humans but also to aid the transport of diclofenac to the epidermis.[150] In addition, when applied

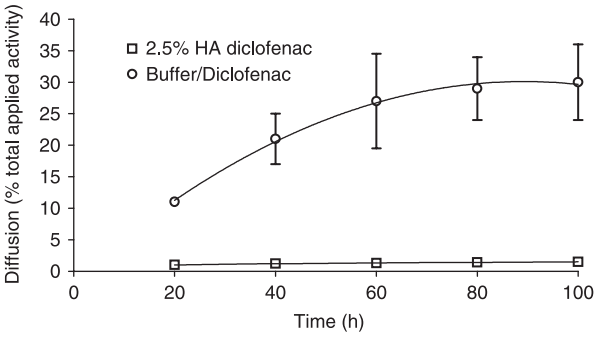

fig. 3 Diffusion of ^{14}C diclofenac through the human epidermal sheet with and without hyaluronic acid (HA) (2.5%) (values represent mean ± SD, $n = 5$, HA diclofenac error bars lie within the data point and therefore not show).

topically to a mouse model of chronic granuloma, diclofenac formulated in HA significantly inhibited vascular development when compared with vehicle alone or diclofenac formulated in a cream indicating that a depot of drug is being formed in the presence of HA. The 3% diclofenac in 2.5% HA gel formulation was also effective in reducing the mass and vascular volume after granuloma formation and in addition, it was capable of halting the development of subcutaneous Colon-26 tumours in syngeneic BALB/C mice after 12 days of topical application (see fig. 4).[151,152] These studies collectively imply that HA's influence on the localized delivery of drugs to the skin can be dependent upon the species of skin used, with the drug depot forming in deeper layers of mouse skin (dermis) compared with humans (epidermis).

Despite all of the above data an exact mechanism of action to explain the topical delivery properties of HA, remains to be elucidated. It is known that the degree of hydration of the stratum corneum influences skin permeability. Increased hydration opens the compact substance of the stratum corneum by loosening the dense, closely packed cells thus increasing the permeability to many drugs.[153] Hydrophobic patches and oleaginous formulations are based on such a principle in that they occlude the skin, inhibiting transepidermal water loss and increasing stratum corneum hydration resulting in enhanced percutaneous delivery of the formulated drug. Polysaccharides, in general, are regarded as moisture-control agents,[154] which are the reason for their incorporation in many topical and cosmetic preparations. However, the skin hydration properties of HA are considered to be much higher than other polysaccharides because of its considerable capacity to bind water.[155] Thus, the topical application of HA may result in increased hydration of the stratum corneum and lead to the enhanced topical delivery of a concomitantly applied drug across this skin barrier.

Such properties, however, do not account for drugs formulated in HA being retained in the skin with little systemic absorption, as indicated previously. Browne *et al.*[150] have reported the surprising movement of HA into the keratin, epidermal and dermal layers of mouse and human skin, with radiolabelled HA apparently being absorbed rapidly from the surface of the skin and into the epidermis. There are three possible factors that may be involved in this behaviour. First, the presence of underlying skin HA receptors[156] may direct the localization of applied HA. Second, the specific structure of hydrated HA and the presence of a hydrophobic area may enable the absorption of this macromolecule across membranes.[157] In turn, the localization of HA can then influence the topical delivery of the drug. Finally, the most favoured explanation, the increased hydration of the surface layers of the skin in the presence of HA not only enhances drug absorption across the stratum corneum, but also facilitates the retention of drug within the more hydrated epidermal layers (possibly by exposure of potential drug binding sites) and in doing so decreases drug diffusion into the lower skin layers. The latter mechanism is based on a more general HA effect and does not necessarily rely on the colocalization of drug and HA.

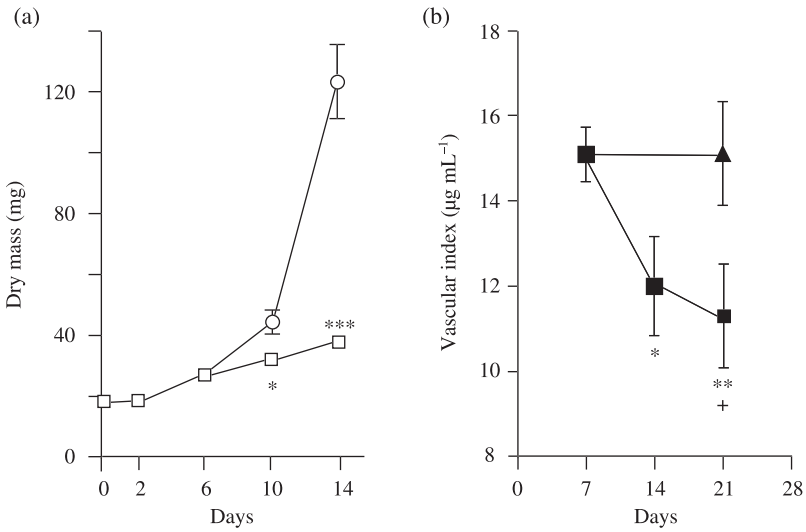

fig. 4 (a) The inhibition of tumour growth and angiogenesis by topical 6 mg/kg diclofenac in 2.5% hyaluronic acid (HA) (□) compared with 0.1 mL aqueous cream BP (○). Results are mean ± SE; $n = 8$–10 per group. $^*P < 0.05$; $^{***}P < 0.0001$ vs. control. Reprinted with permission from Seed *et al.*[152] (b) The regression of the granulomatous tissue neovasculature induced by the daily topical application of HYAL EX-0001 to the established 7-day-old murine chronic granulomatous air pouches. The vascularity was assessed after a further 7 and 14 days. The vascularity index is expressed as μg carmine red per mg dry granulomatous tissue. Points represent mean ± SEM of 0.1 mL HA applied alone (▲) and HYAL EX-0001 (■) ($n = 15$, $^*P < 0.05$, $^{**}P < 0.02$ compared with 7-day control; $^+P < 0.05$ compared with HA control at day 21). Reprinted with permission from Alam *et al.*[151]

Conclusions

Whatever the explanation for its mode of action the fact remains that the inclusion of HA in a topical formulation offers clear and unique potential in the delivery and localization of drugs to the skin. As in the case with diclofenac in Solaraze®, the presence of HA enables the drug to penetrate the outer skin barrier and then form a reservoir or depot in the epidermis, limiting its systemic absorption. Such localization would be desirable for the topical use of many drugs including corticosteroids, immunosuppressants, antihistamines, anaesthetics, retinoids, sex hormones, pediculicides, rubifacients, antifungal, antibacterial, antiparasitic and antiviral agents. Such an effect would be especially advantageous for the delivery of cytotoxic agents for the treatment of other skin cancers or psoriasis, where the adverse side-effects of the drugs when delivered systemically can cause considerable problems.

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