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Hexylresorcinol: Providing Skin Benefits by Modulating Multiple Molecular Targets

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Background

Hexylresorcinol (HR) is an alkylresorcinol (AR), a type of phenolic lipid, having an n-hexyl chain attached to the 4 position of the 1,3-dihydroxybenzene ring (Figure 7.1). It can be synthesized by reacting resorcinol with hexanoyl chloride in the presence of Lewis acid catalyst. The resultant intermediate, hexanoylresorcinol, is then reduced to hexylresorcinol. Subsequent purification of HR is attained by, e.g., crystallization using suitable solvent(s).

Hexylresorcinol is an ingredient that has attained GRAS (Generally Recognized as Safe) status and is effective as an anti-browning agent for prevention of melanosis in shrimp.[1] HR has also been shown to be a very effective inhibitor of surface browning on many fresh-cut fruits, such as apples, pears, mangoes, etc.[2,3] When combined with ascorbic acid, it has a synergistic effect in the prevention of browning. Here, ascorbic acid reduces quinones generated by polyphenoloxidase while HR specifically interacts with polyphenol oxidase, and renders it incapable of catalyzing the enzymatic reaction.[4,5] A post-cutting dip of HR, ascorbic acid, and calcium lactate was found to extend the shelf-life of pear slices from 15 to 30 days.[6] Similarly, Red Delicious apple slices treated with an anti-browning dip (HR, isoascorbic acid, N-acetyl cysteine, and calcium propionate) and held at 5°C maintained visual quality for five weeks.[7] Although sulfites are more commonly used in controlling browning of foods, HR has several advantages over sulfites, including its specific mode of inhibitory action, effectiveness at low concentrations, inability to bleach preformed pigments, and chemical stability.

From a human physiological and biological perspective, HR is perhaps one of, if not the most studied and well-known AR. It is reported to have anesthetic, antiseptic, and anthelminitic properties[8] and can be used topically, e.g., on small skin infections or as an ingredient in a consumable carrier, e.g., throat lozenges.[9] As a throat lozenge it manifests both antiseptic and local anesthetic effects: its antiseptic action killing the bacteria that may be associated with a sore throat, while its anesthetic action helps relieve the pain associated therewith. Here, the action of sucking the lozenge allows the active ingredient to work in the area of the discomfort, and also helps to coat, lubricate, and soothe the irritated throat tissue.

ARs are also found in nature; alkyl chains C17:0–C25:0 attached to 5 position are abundant in wholegrain wheat and rye.[10] ARs are reported to have antitumor, antibacterial, antifungal, and antiparasitic activities. These effects of ARs were attributed to membrane-modulating effects due to interactions of their alkyl tails with phospholipids and/or proteins and to antioxidant effects of the phenolic hydrogen.[11]

HR has a long history of human use. The earliest citation to its use in humans appears to be that of Dr. Veader Leonard and his associates at the Johns Hopkins School of Hygiene and Public Health in Baltimore, Maryland.* As reported, Dr. Leonard and his associates were looking for a "perfect" antiseptic that was deadly to germs but harmless to man. In the course of their efforts, they found that HR possessed over 50 times the germ-killing power of pure carbolic acid.

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[*] Cited by *Time* Magazine, Medicine: Hexylresorcinol, Monday February 23, 1925; obtained from http://content.time.com/time/magazine/article/0,9171,719908,00.html.

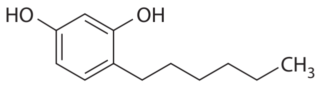

FIGURE 7.1 Structure of hexylresorcinol (HR).

Despite its long history of use as an antiseptic, anesthetic, and anthelminitic, it is only recently that its use and benefits as an active ingredient for skin care applications has been realized. In 2007, Sytheon Ltd. introduced HR as a cosmetic/skin care additive for use as a skin protectant having skin lightening and even-toning properties under the trade named Synovea® HR.[12] Its utility in this application is believed, in part, to arise from the ability to attain highly purified HR (>99%, typically ranging from 99.5% to 99.9%) which is essentially free of resorcinol, a known skin irritant, and hexanoylresorcinol, the intermediate.[12]

Focusing on the human physiological and biological properties of HR, attention will now be given to its antimicrobial, antitumor, anti-aging, and skin lightening/even-toning properties in particular.

Antimicrobial

On July 22, 1991, the U.S. Food and Drug Administration (FDA) published the *Tentative Final Monograph* for antimicrobial drug products—first aid antiseptics[13]—in which HR was identified as a category I antimicrobial ingredient. HR has been used extensively in antibacterial mouthwash and, as noted above, throat lozenges. Antibacterial mouthwashes are effective due to the presence of HR, which inhibits the growth and proliferation of microorganisms found in the mouth: thus preventing caries and ameliorating infectious conditions. As a side benefit, mouthwashes having HR also prevent or reduce halitosis, which, in many instances, is generated or arises from various microorganisms in the mouth. As noted above, lozenges such as Strepsils® Extra Blackcurrant Lozenges containing HR possess both antiseptic properties to fight bacterial throat infections and anesthetic properties to soothe and numb throat pain.

The emergence of pathogenic bacterial strains resistant to currently available antimicrobial agents continues to be a universal problem of ever increasing importance.[14] Extensive efforts have been undertaken to validate new target enzymes for antimicrobials; however, these have met with little success,[15] with the majority of successful drugs inhibiting just a handful of cellular processes. To date, one of the most successfully exploited drug targets has been the DNA topoisomerase (topo) class of enzymes. For example, recent studies by Taylor et al. have shown that HR has good DNA topoisomerase inhibitory activity (IC_{50} 30 μM), which compares well with m-Amsacrine (30 μM) and Purpurin (40 μM).[14]

Chaudhuri has also demonstrated excellent antimicrobial activities of HR in an, as yet, unpublished work. Specifically, Chaudhuri conducted an evaluation to assess the minimum inhibitory concentration values (MIC in μg/mL) of HR against various organisms relevant to personal care applications in accordance with U.S. Pharmacopeia's Compendia Products procedure for Category 2 (USP 26-87 pp. 2022–2026). The results are given in Table 7.1.

In addition, Chaudhuri conducted a challenge test using 0.5% (w/w) level of HR in a lotion. The results showed a 3-log reduction of fungi, but not the desired reduction of bacteria. Nevertheless, it appears that HR can act as a synergist in combination with existing commercial antimicrobial ingredients.

Antitumor

HR has also found potential utility as an antitumor agent, particularly in relation to differentiation therapy. The signaling pathways related to cell differentiation and senescence fail to function properly in malignant tumor cells. As a result, tumor cells exhibit uncontrolled and invasive growth. Differentiation therapy is currently being considered as one of the key emerging techniques for the treatment of cancer.[16] Kim et al. have recently shown that HR induces the differentiation of SCC-9 via the modulation of the E2F-mediated signaling pathway and suppressed the growth of SCC-9 cells in a dose-dependent

TABLE 7.1

Minimum Inhibitory Concentration Values (MIC in µg/mL) of HR against Various Organisms

Organisms	MIC Value (µg/mL)
Bacteria	
E. coli	2.5
S. aureus	4.5
S. epidermidis	4.5
Streptococcus	1.5
Lactobacillus	4.0
P. gingivalis	1.0
P. acne	25 to 50
Fungi	
Aspergillus niger	0.3
Candida albicans	3.5

manner.[17] In this study, the authors also found that HR increased the expression of the epithelial cell differentiation markers involucrin and keratin 10.

Following on the foregoing, Kim and Choi have further demonstrated that HR dose-dependently induced SCC-9 cell apoptosis as determined by caspase-3 activity, annexin V expression, as well as by scanning and transmission electron microscopy.[18] HR was shown to inhibit intracellular calcium oscillation in both SCC-9 cells and normal human dermal fibroblasts. HR-induced apoptosis was partly reversed by calcium channel blockers. Additionally, HR reduced the tumor mass formed by SSC-9 cell implantation in BALB/cAnNCrj-nu/nu mice and mass size reduction was also partly reversed by the concomitant application of calcium channel blockers. The results of this study suggest that HR is providing strong antitumor effects by inhibiting calcium channel oscillation and inducing apoptosis.[18]

Finally, the resistance to chemotherapy is very important in the prognosis of tumors. Transglutaminase-2 (TG-2) mediated chemotherapy resistance has been widely reported. TG-2 is overexpressed in many cancers such as breast cancer,[19] malignant melanoma,[20] and glio-blastoma[21] and facilitates tumor spread and metastasis. Recently, Kim et al. reported on their finding of an inhibitory effect on TG-2 enzyme activity by HR.[17] Additionally, when the authors compared the performance of a mixture containing 5 µg/mL HR and 5 µg/mL cisplatin (a common chemotherapy drug) to 10 µg/mL of cisplatin alone on tumor cells, they found the application of the mixture resulted in significantly lower tumor cell viability than the cisplatin alone ($p < 0.05$). Accordingly, it is believed that HR has a synergistic effect on KB cell death when employed in combination with cisplatin. The authors also examined the effects of the combination of cisplatin and HR on oral mucosal melanoma (OMM) using cultured primary OMM cells in a tumor xenograft model. According to their findings, the combination resulted in fewer metastases and longer survival than cisplatin-only treatment in the OMM xenograft model.[22] Based on these findings, it is now believed that HR could be a viable candidate as a chemotherapeutic agent in the treatment of cisplatin resistant tumors.

Anti-Aging

Recent findings also demonstrate the influence or impact of HR on a variety of processes involved in skin aging and damage, including oxidation, inflammation, and glycation.

Antioxidant

Although HR would not typically be expected to be an effective conventional antioxidant (radical and/or non-radical quencher) based on its structure[23]; it may still be considered an antioxidant in a broader sense because its metabolites, such as, 1,2,3-trihydroxy- and/or 1,3,5-trihydroxy-4-hexylresorcinols, can have potent radical and non-radical quenching activity.[11] Antioxidant activity may also be attributed to HR, though indirectly, as a result of its ability to stimulate the cell protectant glutathione and various antioxidant defense enzymes such as glutathione peroxidase and glutathione reductase.[24] For example, Yen et al. have demonstrated that HR has a protective effect against oxidative DNA damage in human lymphocytes induced by hydrogen peroxide.[24]

Inflammation

Inflammation is a complex physiological process and the role of transcription factor NF-kappaB in the inflammatory response has been well documented.[25,26] NF-kappaB is activated by numerous stimuli and once fully activated participates in the regulation of various target genes in different cells to exert its biological functions. NF-kappaB has often been referred to as a central mediator of the immune response since a large variety of bacteria and viruses can lead to the activation of NF-kappaB, which in turn controls the expression of many inflammatory cytokines, chemokines, immune receptors, and cell surface adhesion molecules.

Recently, Kim et al. reported that HR inhibited NF-kappaB phosphorylation.[27] Independently, Johnson & Johnson scientists found that HR has a stimulating effect on collagen and elastin synthesis through NF-kappaB inhibition.[28] Based on these findings, and the fact that inflammation has a significant effect on skin aging, it is anticipated that HR would provide an anti-inflammatory as well as anti-aging effect on skin when applied topically.

Glycation

Glycation is the term used for a class of non-enzymatic reactions that occurs between sugars, such as glucose or ribose, and proteins and lipids, including, e.g., the reaction between the nucleophilic amino group of proteins and a reducing sugar wherein the sugar becomes bonded to the protein. Glycation is the first of a series of reactions by which advanced glycation end-products (AGEs) are formed: the subsequent reactions include Schiff base reactions, Amadori reactions, and Maillard reactions. Though not all glycation reactions lead to AGEs, physiological and biological conditions will shift the reactions to favor or disfavor their formation.

AGEs are heterogeneous compounds which adversely affect nearly every type of cell and molecule in the body and lead to human pathological conditions. AGEs are thought to be one of the key factors in skin aging. For example, studies of collagen glycation using skin equivalents[29] found a number of changes including modified fibroblast shape and distribution, enhanced extracellular matrix molecules and the dermal-epidermal junction zone, and increased collagenase activity. Additionally, AGE treatment has been found to reduce various biomarkers of skin aging, including those noted above, as well as increased NF-κB activation and cytokine expression.[30] Exposure of AGEs to ultraviolet (UV) light generates reactive oxygen species (ROS) in extracellular matrix.[31]

Turning to HR, it has been reported to have an inhibitory effect on the formation of Maillard reaction products (*in vitro* using glucose and cysteine).[32] Given the importance of Maillard reactions in the formation of AGEs, it is expected that HR, when applied topically, would provide skin benefits due to its anti-glycation/anti-AGE property.

Melanin and Melanogenesis

Pigmentation

Skin color is one of the most important physical traits of humans because it affects so many aspects of our health and social well-being. Skin color is also one of the best examples of evolution by natural selection acting on the human body. Anthropologic studies have provided us with two important facts: the earliest *Homo sapiens* had dark skin, rich in protective melanin, and small groups of “modern” humans dispersed out of the African tropics into less intensely sunny parts of Africa, Eurasia, and the Northern Hemisphere. Over time, these latter groups of humans underwent genetic changes leading to the loss of melanin pigmentation.[33] Skin color is one of the most conspicuous ways in which humans vary and has been widely used to define human races. Unfortunately, skin color is also a determinant of human interaction and destinies.

Human skin coloration is both adaptive and labile as evidenced by the multitude of different skin colors of humans around the world. Though humans are often characterized as having black, white, red, or yellow skin, no one is actually any of these colors, as these are commonly used terminologies that do not reflect biological reality.[34] Skin coloration in humans arises from a complex series of cellular processes involving the synthesis and transfer of a pigment, melanin, which, besides being responsible for skin color and tone, is the key physiological defense against sun-induced damage, such as sunburn, photo-aging, and photo-carcinogenesis. The melanin itself is formed by a sequence of reactions in which tyrosine is oxidized to dopa and then dopa is subsequently oxidized to melanin. These reactions are catalyzed by the enzyme tyrosinase. The formation of melanin, also known as melanogenesis, is carried out in melanosomes, organelles of that population of cells known as the melanocytes, which are located in the lower part of the epidermis. The melanosomes containing the melanin are subsequently transferred from the melanocytes to the neighboring cells, the keratinocytes, which then transport and distribute the melanin to the upper layers of the skin.[35]

Skin coloration is primarily regulated by the amount and type of melanin synthesized by the melanocytes[36,37]; however, additional and equally contributing factors include (a) the efficiency of the transfer of the melanosomes, hence the melanin, from the melanocytes to the neighboring keratinocytes, a process that occurs with the help of E-cadherin, an adhesion protein, and (b) the subsequent distribution and degradation of the transferred melanosomes by the recipient keratinocytes.[38] Although a number of factors can influence the formation of melanin, exposure of the skin to UV light markedly influences and increases the amount and rate of melanin production, most often producing a further darkening of the skin or a "tan." Sun exposure as well as hormones and other environmental and physiological factors can also lead to more localized skin pigmentation, e.g., age spots, melasma, and freckles, owing to an overabundance of melanin production in the afflicted areas of the skin.

Skin Lightening/Even-Toning

For a substantial segment of the human population, one's natural skin color is not satisfactory and efforts are undertaken to modify it. For example, in North America, Europe, and Australia, many endeavor to enhance skin pigmentation through tanning, whether natural (induced melanin production) or artificial (dyes). Others, especially certain Asian cultures, endeavor to prevent skin coloration and/or seek to actually lighten their natural skin color. Additionally, skin lightening efforts are oftentimes undertaken to address or eliminate age spots, melasma, and freckles, or to obtain even-toning effect with one's skin.

HR has been found to have a significant skin lightening effect due to a strong inhibitory effect on tyrosinase and peroxidase and a stimulatory effect on glutathione and E-cadherin syntheses. It is believed that the HR bind to tyrosinase directly and inhibits its enzyme activity.[39] Additionally, having observed that fragments of DNA can stimulate melanin synthesis, it seems that the reduction of DNA damage by HR, as previously noted, may also be responsible for inhibition of melanin synthesis.

Chen et al. found that HR inhibits both the monophenolase (tyrosine to DOPA) and diphenolase (DOPA to DOPAchrome) activity of mushroom tyrosinase.[39] Its activity or mode of action, as shown by a kinetic analysis, is as a competitive inhibitor. More recently, Chaudhuri demonstrated that HR is a far superior tyrosinase inhibitor (using both tyrosine and DOPA substrates) than the three commercially available skin lighteners: hydroquinone, kojic acid, and licorice extract.[12] Concurrently, HR was found to provide superior inhibition of peroxidase activity of HR as compared to the three skin lighteners tested as well.[12] This study also showed that at 10 μg/mL use level, HR had inhibitory effects on extracellular and intracellular melanin production by 75% and 36%, respectively, when compared with placebo. Related studies using B16 melanoma cells showed that their growth rate was not significantly altered in the presence of HR during a 72 h incubation period, indicating that the HR-induced regulatory effects on melanogenesis of melanoma cells occurred without affecting cell proliferation.[12]

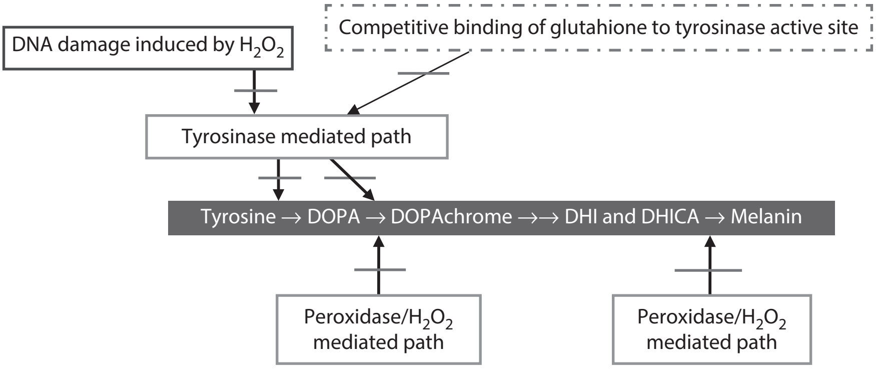
Competitive binding of glutahione to tyrosinase active site
DNA damage induced by H_2O_2
Tyrosinase mediated path
Tyrosine → DOPA → DOPAchrome →→ DHI and DHICA → Melanin
Peroxidase/H_2O_2 mediated path
Peroxidase/H_2O_2 mediated path

FIGURE 7.2 Sites in the melanogenesis pathway in melanocytes inhibited by HR. DOPA = 3,4-dihydroxyphenylalanine; DOPAchrome = 5,6-dioxo-2,3,5,6-tetrahydro-1H-indole-2-carboxylic acid; DHI = 5,6-dihydroxyindole; DHICA = 5,6-dihydroxyindole-2-carboxylic acid.

Literature data shows that low glutathione levels relates to the deposition of melanin in the skin of humans and other animals, whereas high glutathione levels inhibit melanogenesis.[40,41] It is also reported that glutathione depletion increases tyrosinase activity in human melanoma cells.[42] UVA irradiation induces the immediate loss of reduced glutathione (GSH) in both melanocytes and keratinocytes.[43] Recently, Matsuki et al. have reported that glutathione has a dose dependent inhibition on melanin synthesis in the reaction of tyrosinase and L-DOPA.[44] Since HR has a dose dependent increase effect on glutathione synthesis,[24] it can be concluded that HR also inhibits melanogenesis by stimulating glutathione synthesis.

It has also been found that human skin exposed to UVB irradiation with a dose of 2 MED manifests a significant increase in the expression of Endothelin-1 (ET-1) and tyrosinase mRNA signals five days after irradiation.[45,46] In these studies, low levels of ET-1 secreted by keratinocytes in response to UVR was shown to down-regulate E-cadherin in melanocytic cells. ET-1 is a potent down-regulator of E-cadherin in human melanocytes and also melanoma cells.[46] An independent study by Chaudhuri has shown a 15-fold up-regulation of CDH-1 gene coding for E-cadherin as compared to a control in UVB irradiated normal human keratinocytes treated with HR. In light of the foregoing, it is quite tempting to propose that HR also reduces UV-induced hyperpigmentation by modulating E-cadherin.

Eller et al. have shown that DNA damage after UV irradiation enhances UV-induced melanization.[47] They have also shown that the addition of small DNA fragments to un-irradiated pigment cells *in vitro* or *in vivo* to guinea pig skin induces a pigment response indistinguishable from UV-induced tanning. Based on the study reported by Yen et al. which found that HR reduces DNA damage induced by hydrogen peroxide[24]; it is quite conceivable to conclude that HR also inhibits melanin synthesis by reducing DNA damage.

Taken together, the data generated by the present author and available in the literature strongly suggests that HR provides for a reduction in melanin production by modulating multiple sites in the melanogenesis pathway in melanocytes. This pathway and the sites of action of HR (shown by arrows) are portrayed in Figure 7.2.

Human Clinical

In addition to the multitude of *in vitro* and non-human *in vivo* studies on the effects of HR on skin mechanisms and physiology, many of which are discussed above, there are a number of human clinical studies that have been reported. Chaudhuri has reported on the skin-lightening efficacy and safety of a lotion containing 0.5% HR compared with a 2% hydroquinone lotion.[12] This was a single-center study of 15 subjects who applied the lotions twice a day, morning and evening, for eight weeks. The test sites, as well as untreated sites on each forearm, were evaluated for skin color change as represented by the change in

TABLE 7.2

Human Clinical Study: Skin Lightening Effects of HR vs. Hydroquinone

Skin Lightening Active	Week	L-value	% Improvement Based on L-Value	ITA°	% Improvement Based on ITA°
0.5% HR	0	56.88	–	19.09	–
	4	58.82	3.4	24.34	27.5
	8	59.54	4.7	26.26	37.6
2.0% Hydroquinone	0	57.10	–	20.72	–
	4	58.95	3.2	24.67	27.7
	8	59.54	4.3	27.22	38.4

L values and ITA° (Individual Topology Angle—COLIPA SPF test method). Changes were measured by chromometric measurement. The results of this study are presented in Table 7.2 where the delta represents the percent change in skin color from the baseline coloration of the untreated skin. According to these results, the HR lotion was found to provide a comparable, statistically significant (p value ≥ 0.001) change in skin color to that attained with the like composition containing 2% hydroquinone. Such results are consistent with a “lightening” of the skin. No adverse effects were noted for either lotion over the test period.

Another clinical study was carried out by the present author using 1% HR lotion for treating individuals having hyper-pigmented spots. Eighteen volunteers between the ages of 42 and 60, of which there was a mix of Caucasian (10), Asian (7), and Hispanic (1), applied the 1% HR lotion twice daily in an amount sufficient to cover their upper arm, including specifically the targeted hyper-pigmented spots. The treated skin of each volunteer was evaluated and the percent reduction in pigmentation of the treated skin was determined using ITA degree. When compared to the coloration on day zero (pre-application), it was found that the treated hyper-pigmented spots showed a statistically significant reduction of 39% and 60% in pigmentation at four and eight weeks, respectively. On the other hand, the treated area of the skin surrounding the hyper-pigmented spots showed only marginal changes when compared to day zero (pre-application). Based on this study, one could conclude that a 1% HR lotion provides an even-toning effect.

Chaudhuri also investigated the potential for HR to show a synergistic effect when used in combination with other skin lightening ingredients. In this study, a lotion containing 2% by weight of a commercial blend of HR (25%) and ethyl linoleate (EL, 25%) in caprylic/capric triglycerides (trade name Asyntra® SL) was applied twice daily for a period of four weeks to the whole face of 15 volunteers. As in the previous studies, skin color was determined by ITA degrees before and after the treatment. The results of this study demonstrated a statistically significant ($p \leq 0.05$) lightening effect on the natural skin color (initial ITA degree 2.1 vs. 5.8 after four weeks of treatment). The combination was also found to be much more effective than HR alone in lightening one’s natural skin color. The synergy of the ingredients of this blend was subsequently confirmed by carrying out an *in vitro* study using B16 melanoma cells. In that study, referenced in the preceding section, an almost identical level of reduction in melanin synthesis was observed using the HR-EL blend (contains only 25% HR) as compared to the use of HR alone. Although the full mechanism is unknown, it has been reported that EL is hydrolyzed to linoleic acid (LA) *in vivo*,[48] which has been reported to degrade post-translational tyrosinase and is also involved in the increase in cell turnover.[49] Therefore, it can be presumed that, in addition to the synergistic effect with HR, EL is also providing all of the skin benefits resulting from its conversion to LA.

Independently, Makino et al. reported on a single-center, double-blind comparison clinical study of 18 subjects in which the efficacy of a HR-containing (included several other skin lightening ingredients) cream for reducing ultraviolet-induced hyperpigmentation was evaluated.[50] Test sites were irradiated with 1.0, 1.5, 2.0, and 2.5 minimal erythema doses. After five days during which pigmentation was allowed to develop, the HR-containing product or a 4% hydroquinone cream was applied to the respective test sites, once daily for four weeks. Chroma meter measurements (L* brightness) and standardized digital photographs were taken of the test sites twice a week. According to their findings, areas treated with the HR-containing product produced greater increases in L* brightness as compared with those treated with the 4% hydroquinone cream. This study demonstrates that a product designed to affect multiple pathways of melanogenesis and melanin distribution may provide an additional treatment option beyond hydroquinone for hyperpigmentation.

In another study, Fantasia et al. reported on a 12-week, double-blind, split-face, randomized clinical study in which an HR-containing lotion was applied to 41 subjects using a round-robin approach against the vehicle, which was applied to 40 subjects, all subjects being women from 35 to 59 years of age.[51] Subjects were selected specifically for overall photo-damage, fine lines, and mottled pigmentation. Each product was applied once daily to the designated half side of the face. Evaluation was done at baseline and after 2, 4, and 12 weeks of treatment using a 1–9 scale for photo-aging parameters. The findings from this study revealed that the HR-containing lotion performed significantly well as compared to the placebo on several key anti-aging clinical parameters, such as tactile roughness, radiance, mottled pigmentation, crow's feet fine lines, and overall photo-damage. Significant clinical benefits were observed as early as two weeks, with progressive improvements at weeks 4 and 12. Such results are consistent with the reports by these authors on the stimulatory effect of HR on collagen and elastin synthesis through NF-kappaB inhibition.

Roure et al. reported on a double-blind, randomized, placebo controlled study on healthy women volunteers aged over 45 years having wrinkles, sagging, spots, and a dull complexion on the face using a HR and ascorbic acid-2-glucoside-containing formulation.[28] Forty-two volunteers applied the product and placebo in a split-face mode, twice a day for 12 weeks. Clinical grading and self-assessment of the signs of aging, cutometric, and colorimetric measurements were performed at baseline and after eight and 12 weeks of application. After eight weeks of daily application, a significant improvement of the wrinkles (crow's feet: +17%, under eye: +19%, cheek: +10%), fine lines (crow's feet: +9%, under eye: +13%), brown spots (intensity: +11%, number: 8%), firmness (+26%), and skin texture (complexion: 18%, homogeneity: +26%, softness: 21%, smoothness: 32%) was observed with the product versus baseline as well as placebo. These changes were even greater after 12 weeks of product application.

Finally, in a 12-week, full face, double-blinded, randomized controlled study in Chinese subjects, Johnson & Johnson scientists have shown significant improvement in key pigmentation-related parameters without detecting any product-related adverse events.[52] Out of the total study population of 65, 32 subjects using the lotion containing HR product and 31 subject using the vehicle (without HR) completed the 12-week study. Two subjects from the vehicle group were discontinued for non-product related reason. Application of HR containing product resulted in significant clinical improvement (statistically significant vs. placebo; $p < 0.05$) on overall skin lightening (% of subjects showing improvement: 88%), appearance of spots on the cheeks (88%), overall contrast between spots and surrounding skin (100%), and overall pigmentation size (97%) with the vehicle at 12 weeks. Changes were determined by dermatologist grading, digital photos and UV images. These authors further demonstrated that de-pigmenting effect of HR is not associated with toxicity in melanocytes and is reversible; confirming Chaudhuri's earlier observation.[12] In contrast, the inhibition of melanin production, as expected, failed to recover in melanocytes treated with hydroquinone. These findings further validate the potency of HR to modulate skin pigmentation, and its safety and tolerance for topical application.

These studies show that HR has a plurality of strong, beneficial effects on skin physiology and conditioning, including, but not limited to skin lightening, even toning of color, and anti-aging effects.

Formulation

Having demonstrated the benefits of HR as a skin treatment, HR also benefits from an ease of use in terms of formulating products, especially skin care and cosmetic products. This is especially so at use levels of 0.5%–1.0% (w/w) of HR in the finished formulation, which have been found to provide effective products.

HR is highly soluble (>20%) in a number of cosmetic and skin care ingredients and carriers including caprylic/capric triglycerides, isosorbide dicapylate, ethyl linoleate, ethoxydiglycol, and other high polarity esters; non-ionic solubilizers; glycerol; and a wide range of glycols. PEG-400 was found to be an excellent solubilizer of HR (>50%). Such solubility provides a wide range of options to develop elegant formulations with HR. HR can also be easily combined with other skin lightening ingredients, such as niacinamide, ethyl linoleate, acetyl-glucosamine, ascorbyl-2-glucoside (not magnesium and sodium ascorbyl phosphate as these two requires slightly basic formulation pH), standardized plant extracts, such as *Phyllathus emblica* (trade named Emblica®), *Terminalia chebula* (trade named Synastol® TC), etc., thereby opening the door to a multitude of synergistic products to make HR even more effective.

Generally, the pH of the formulation to which HR is added must be acidic due to the presence of phenolic OH in HR. Similarly, it is found that the use of non-ionic emulsifiers is preferable to anionic types. For the preparation of serum or transparent gels, the use of non-ionic solubilizers having high HLB values like PEG-40 hydrogenated castor oil, Laureth 23, Polysorbate 20, and Polysorbate 80 are recommended.

Finally, the addition of HR to various formulations may cause a drop in overall viscosity of the product. In such cases, anionic (such as Xanthan gum, Carbomers) or neutral thickeners (such as Cellulosics) can be added for maintaining and/or restoring the desired viscosity. If needed, low levels 0.1%–0.2% of propyl gallate (SynoxylÒ PGL) can be used to minimize color shift over time.

Conclusion

Although the present author endeavored to identify all relevant articles and dates, it is nonetheless acknowledged that some will have been overlooked, for which the author apologizes. Nevertheless, this chapter provides ample evidence of the complex physiological actions of HR and its ability to modulate different biological processes and pathways through multiple molecular targets. Key targets include those associated with skin pigmentation, inflammation and inflammatory responses, extracellular matrix proteins, and the like. Such activity clearly points to the realized and yet to be realized significance of HR in providing a number of skin benefits of commercial interest. Already, commercial use of HR in a wide variety of skin care and treatment products has begun and is expanding. Additionally, further work exploring and fleshing out the impact of HR on tumors will likely lead to the establishment of HR as a key ingredient in combination therapy. In summary, it is clear from the current literature that HR has numerous targets, and provides its biological effects through simultaneous modulation of these targets.

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