



University of
Massachusetts
Amherst

HORMESIS [BIOLOGICAL EFFECTS OF LOW LEVEL EXPOSURES (BELLE)] AND DERMATOLOGY

Item Type	article;article
Authors	Thong, Haw-Yueh;Maibach, Howard I
Download date	2025-05-19 08:32:35
Link to Item	https://hdl.handle.net/20.500.14394/20529

HORMESIS [BIOLOGICAL EFFECTS OF LOW LEVEL EXPOSURES (BELLE)] AND DERMATOLOGY

Haw-Yueh Thong, MD, MS, Howard I. Maibach, MD □ Department of
Dermatology, University of California, School of Medicine, San Francisco, CA

□ Hormesis, or biological effects of low level exposures (BELLE), is characterized by nonmonotonic dose response which is biphasic, displaying opposite effects at low and high dose. Its occurrence has been documented across a broad range of biological models and diverse type of exposure. Since hormesis appears to be a relatively common phenomenon in many areas, the objective of this review is to explore its occurrence related to dermatology and its public health and risk assessment implication. Hormesis appears to be a common phenomenon in *in-vitro* skin biology. However, *in vivo* data are lacking and the clinical relevance of hormesis has yet to be determined. Better understanding of this phenomenon will likely lead to different strategies for risk assessment process employed in the fields of dermatologic toxicology and pharmacology. We believe that hormesis is a common phenomenon and should be given detailed consideration to its concept and its risk assessment implications, and how these may be incorporated into the experimental and regulatory processes in dermatology. The skin, with its unique characteristics, its accessibility, and the availability of non-invasive bioengineering and DNA microarray technology, will be a good candidate to extend the biology of hormesis.

Keywords: Hormesis, biological effects of low level exposures (BELLE), biphasic dose-response relationships, dose response, dermatology, keratinocytes, melanocytes, fibroblasts, hair follicle, tumor cell lines, toxicology, pharmacology

INTRODUCTION

Biphasic dose response, namely a low-dose stimulatory and a high-dose inhibitory response, also called hormesis, or biological effects of low level exposures (BELLE) in the field of toxicology, has been noted in a wide range of biological model systems from immunology to cancer biology (Calabrese and Baldwin 2001, 2003; Calabrese 2005a, 2005b). Calabrese has been the mainstay in bringing the attention of the scientific community to this interesting and a not uncommon phenomenon (Calabrese and Baldwin 2001, 2003; Calabrese 2005a, 2005b). As noted by Calabrese, the quantitative features of the hormetic-like biphasic dose response were remarkably similar with respect to the amplitude of the stimulatory response, the width of the stimulation, and the relationship of the maximum stimulatory response to the zero equivalent point (ZEP, i.e. threshold). Typically, the low-dose hormetic biphasic dose response

Address correspondence to Howard I Maibach, MD, Department of Dermatology, University of California, School of Medicine, BOX 0989, surge 110, San Francisco, CA 94143-0989, USA; Tel: +1 415 476 2468, Fax: +1 415 753 5304, e-mail: maibachh@derm.ucsf.edu

stimulation is modest, with maximum stimulation between 30 to 60% greater than controls, and has a rather similar appearance in different cell types with various chemicals (Calabrese 2005b). Most stimulatory ranges were less than 100-fold (averages 10- to 20- fold) measuring back from the ZEP. The low-dose stimulatory response often occurs following an initial disruption in homeostatis and appears to represent a modest overcompensation response. It is believed that the modest stimulatory responsiveness is due to the result of a compensatory process that “slightly” overshoots its goal of the original physiological set-point, ensuring that the system returns to homeostasis without unnecessary and excessive overcompensation (Calabrese 2001). Therefore, it is important to follow the dose-response relationships overtime in order to better define its quantitative features. While initial interest focused on the hormetic effects of pollutants and toxic substances on biological systems (Calabrese and Baldwin 1997), the interest expanded to include pharmacological agents, phyto-compounds, as well as endogenous agonists (Calabrese 2005b). The hormetic-like biphasic dose response relationships appears to be highly generalizable; that is, such responses do not appear to be restricted by biological model, endpoint, or chemical/physical stressors (Calabrese 2005b).

Many investigations attempted to assess mechanisms that could account for the hormetic-like biphasic dose-response relationship. In general, there is no single mechanism that accounts for the plethora of hormetic relationships. Nonetheless, a common molecular tactic by which biphasic dose-response relationships are displayed involves the presence of two receptor subtypes affecting cell regulation, one with high and the other with low affinity for the agonist but with notably more capacity (i.e. more receptors) (Calabrese 2005b). Such an arrangement may lead to the biphasic dose response, with the high-affinity receptor activated at low concentrations, which stimulates DNA synthesis and cellular proliferation; and the low affinity/high-capacity receptor becoming dominant at higher concentrations decreasing the cell proliferative response. This is a general pharmacological mechanism in that it is employed for a large number of receptor-based responses from cancer cells to neutrophil chemotaxis and many others.

This article reviews hormetic effects of various agents on *in vitro* skin biology. Recognition of this emerging biological phenomenon in dermatology should lead to markedly improved integrative assessments of animal/human skin responses to toxic substances, pharmacological agents, as well as endogenous agonists.

EVIDENCES OF HORMESIS IN SKIN CULTURES

Skin is a complex biological model but highly approachable. Models exist for dermatologic research which include animal *vs* human skin

models, *in vitro* vs *in vivo* models, regional variation, stem cell biology and hair follicle biology. Many pharmaceutical preparations in dermatology affect cell regulation. Nonetheless, the FDA sometimes exempts dose justification for dermatologic preparations. As a result, the presence of any hormetic effect might have been missed.

The literature in dermatology indicates that several cell types in the skin provided evidence of hormetic-like biphasic dose/concentration-response relationships. A brief listing of the cell types showing hormetic relationships and the quantitative features of dose responses is presented in Table 1.

MELANOMA AND TUMOR CELL LINES DISPLAY HORMETIC DOSE RESPONSES

Perhaps a more important issue regarding hormesis is its relationship to cancer biology. The existence of hormetic dose responses in many tumor cell lines has been noted and reviewed by Calabrese(2005b). 12 melanoma cell lines (M4Beu, B16, M24, MNT, SK-MEL, H1144, SK-MEL28, Cal 1, Cal 4, Cal 23, Cal 24, Cal 32) have been shown to display hormetic dose responses to various chemicals (guanine or guanosine derivatives, mistletoe extract, salsolinol, tetrahydropapaveroline, dopamine, resveratrol, thrombin and suramin) (Calabrese 2005b). Numerous endogenous agonists, drugs, environmental contaminants, and phytochemicals, some relevant to dermato-toxicology and dermato-oncology, have also been noted to exert hormetic dose responses in various tumor cell lines (Calabrese 2005b). Examples and the proposed mechanistic explanations are listed in Table 2.

HORMESIS AND ITS IMPLICATION IN SKIN AGING

Detailed molecular mechanisms of the hormetic effects on cell aging are being increasingly understood. Studies have shown that repeated mild heat stress (RMHS) has anti-aging effects on serially passaged human fibroblasts throughout their replicative lifespan *in vitro*. Treatment with RMHS at 41° C, for 1 hour twice a week, increased the levels of various chaperones and antioxidant enzymes, increased the phosphorylation-mediated activities of various stress kinases, reduced the accumulation of oxidatively and glycoxidatively damaged proteins, stimulated proteasomal activities for the degradation of abnormal proteins, improved cellular resistance to ethanol, hydrogen peroxide, and UV-B rays. RMHS-treated aging human fibroblasts are also better protected against glucose- and glyoxal-induced growth inhibition and apoptosis. Various hormetic effects of RMHS were also noted on normal human epidermal keratinocytes, including increased replicative life span, increased proteasomal activity, and enhanced levels of Na/K-ATPase pump. (Rattan

TABLE 1. Examples of Hormesis in Cultured Skin

Chemicals and Cell Type	Stimulatory responses	Inhibitory responses	References
Sodium Lauryl Sulfate (SLS) Cultured keratinocytes	SLS (10^{-8} to 10^{-5} M) For 1 h: 36% stimulation For 18h: 12% stimulation For 4 days: ~89% stimulation Subconfluent fibroblast:	> 10^{-5} M	Bloom <i>et al.</i> 1994
Cultured fibroblasts	For 1 h (10^{-5} M): 38% stimulation For 18h (10^{-6} M): 32% stimulation Confluent fibroblast for 1 h (10^{-8} M): 40% Stimulation		
SLS vs retinoic acid (RA) Cultured keratinocytes	0.11×10^{-5} M 1. For 2 h: from 4×10^{-4} (baseline cell acid (RA) count) to a maximum of 8×10^{-4} cells 2. SLS requires a higher molar concentration ($0.75\text{-}3 \times 10^{-6}$ M) to achieve optimal stimulation and the number of responding cells at optimal concentrations is less with SLS than RA.	> 5×10^{-5} M 1. SLS has a very narrow stimulatory range and concentrations that are slightly higher than those that stimulate proliferation are toxic (complete inhibition > 5×10^{-5} M) 2. In contrast, supra-optimal concentrations of RA are much less toxic	Varani <i>et al.</i> 1991
Cultured dermal fibroblasts	1. Similar stimulatory response as keratinocytes at the same doses 2. A greater fibroblast proliferative response to optimal concentrations of RA than to optimal concentrations of SLS.		
Imidazole derivatives (econazole, clotrimazole) Reconstructed human epidermis	5-133 μ M (18 h contact time): biphasic effect on 7-ethoxycoumarin-o-deethylase (ECOD) activity in the epidermis, with induction (60- 70% increase of the basal level) at low concentrations and inhibition (40% of the basal level) at high concentrations	>133 μ M	Cotovio <i>et al.</i> 1996

Table 1 continued on next page

TABLE 1. Examples of Hormesis in Cultured Skin, *continued*

Chemicals and Cell Type	Stimulatory responses	Inhibitory responses	References
Ionizing radiation Cultured human dermal fibroblasts	The plating efficacy reached values significantly above 100% apparent survival at low doses (≥ 40 cGy)	≥ 40 cGy	Smith <i>et al.</i> 1992
	Corticotropin-releasing hormone		
Cultured human sebocytes	Biphasic effect on sebaceous lipid synthesis and up-regulation on the mRNA levels of 3- β -hydroxysteroid dehydrogenase/ Δ^5-4 isomerase (10^{-7} M), but did not affect cell viability, cell proliferation, or IL-1 β -induced IL-8 release		Zouboulis <i>et al.</i> 2002
Arsenite			
Human epidermal keratinocytes, Promyelocytic leukemia cells	Exposed to arsenite from 0.1-40 μ M for 1,3,5 days, cell growth was increased at low doses (0.5 μ M)	>1 μ M	Zhang <i>et al.</i> 2003
Arsenic trioxide (As)			
Keratinocytes, Melanocytes, Dendritic cells, Dermal fibroblasts, Microvascular endothelial cells, Monocytes, T cells	Exposed to for 72h: 1. Sublethal doses of As stimulate cell proliferations. 2. As is toxic at high doses to keratinocytes, fibroblasts, monocytes, and T cells; and toxic at low doses to melanocytes, microvascular endothelial cells and dendritic cells. Peak proliferation: Keratinocytes: 30 μ M; Melanocytes: 0.95 μ M; Dendritic cells: 0.96 μ M; Fibroblasts: 7.6 μ M; Microvascular endothelial cells: 0.95 μ M; Monocytes: 30 μ M	LD ₅₀ : Keratinocytes: 45.5 μ M; Melanocytes: 7.6 μ M; Dendritic cells: 7.6 μ M; Fibroblasts: 187 μ M; Microvascular endothelial cells: 2.4 μ M; Monocytes: 252.7 μ M	Graham-Evans <i>et al.</i> 2003

Table 1 continued on next page

TABLE 1. Examples of Hormesis in Cultured Skin, *continued*

Chemicals and Cell Type	Stimulatory responses	Inhibitory responses	References
Mixture of 4 metals (Arsenic, Chromium, Cadmium, Lead) Human keratinocytes	Exposed for 24h: Lowest mixture dilution (0.0014x of mixtures containing the predetermined LD ₅₀ for each metal: As 7.7µM, Cr 4.9µM, Cd 6.1µM, Pb 100µM) with a total concentration of 0.163µM had a percent viability of 116.6%, clearly above that observed in the single-chemical data. It is likely that this enhancement of cell viability at the lowest mixture level is indicative of the presence of hormesis caused by the mixtures, rather than individual metals	Synergistic cytotoxicity at total concentration of 8-36µM of the metal mixture (a number of mechanisms of toxicity for the different metals may work in concert, overwhelming growth recovery mechanisms)	Gennings <i>et al.</i> 2002
Arsenite (iAs ^{III}), Arsenate (iAs ^V), dimethylarsinic acid (DMAs ^V) Human keratinocytes	1. iAs ^{III} and DMAs ^{III} GS induced an increase in cell proliferation at low concentrations (0.001 to 0.01µM), while at high concentrations cell proliferation was inhibited. 2. pentavalent arsenicals did not stimulate cell proliferation 3. methylated forms of As ^V were more cytotoxic than iAs ^V	Arsenite (DMAs ^{III} GS), methylarsonic acid with glutathione (DMAs ^{III} GS), methylarsonic acid (DMAs ^V) iAs ^{III} : >0.5µM iAs ^V : >1.0µM DMAs ^{III} GS: >>0.05µM DMAs ^V : >0.1µM	Vega <i>et al.</i> 2001
Minoxidil Human epidermal and follicular keratinocytes	0.1 to 10µM (Exposed for 5-8 days): Minoxidil had biphasic effects on the proliferation and differentiation of both epidermal and follicular keratinocytes, stimulating proliferation at micromolar doses, while antiproliferative, pro-differentiative and partially cytotoxic effects were observed with millimolar concentrations	>1 mM	Boyera <i>et al.</i> 1997

Table 1 continued on next page

TABLE 1. Examples of Hormesis in Cultured Skin, *continued*

Chemicals and Cell Type	Stimulatory responses	Inhibitory responses	References
Nitric oxide (NO) donors: Nitroprusside, SIN-1, DETA/NO, SNAP Human keratinocytes, fibroblasts	Four different NO donors at concentrations ranging from 0.01 to 5mM were added every 12 h or 24 h, and cells cultured for up to 3 d in the presence of these compounds: Keratinocytes: 1. a biphasic effect is found with increased proliferation at low concentrations and cytostasis at high concentrations 2. cytokeratin 6 expression is decreased at the lower NO donor concentrations and increased at higher concentrations as an indication of induction of differentiation at higher NO concentrations. Fibroblasts: Cytostasis becomes significant at $\geq 0.25M$ of the NO donor		Krischel <i>et al.</i> 1998
Antioxidants: Ascorbic-2-O-phosphate (Asc2P), Ascorbic-2-O-alpha-glucoside (Asc2G); Pro-oxidants: hydrogen peroxide (H ₂ O ₂) Human keratinocytes	Repetitive addition of Asc2P and Asc2G: cellular life-span of keratinocytes was shown to be extended up to 150% of population doubling levels (PDLs). Pro-oxidants: 20 μM H ₂ O ₂ : extended up to 160% of PDLs, 60 μM H ₂ O ₂ : extended up to 120% of PDLs The longevity of the keratinocytes was suggested to be achieved by slowdown of age-dependent shortening of telomeric DNA rather than by telomerase; telomeres may suffer from less DNA lesions due to the continuous and thorough repression of intracellular reactive oxygen species (ROS), which was realized either by pro-vitamin C such as Asc2P or Asc2G (with antioxidant ability more persistent than Asc) or by 20(M H2O2 which diminished intracellular ROS assumedly through a hormesis-like effect.		Yokoo <i>et al.</i> 2004

Table 1 continued on next page

TABLE 1. Examples of Hormesis in Cultured Skin, *continued*

Chemicals and Cell Type	Stimulatory responses	Inhibitory responses	References
1,25-dihydroxy vitamin D ₃ (1,25(OH) ₂ D ₃) Whole organ cultures of hair follicle	<p>Biphasic dose-response relationships for the effects of 1,25(OH)₂D₃ on the total cumulative growth of hair follicles and hair fibers.</p> <p>At relatively low concentration, growth of follicles and fibers was stimulated, to a maximal extent at 10nM of 52% and 36%.</p> <p>The concentration producing 50% of the maximal response (EC50) for both follicle and fiber growth stimulation were 0.3nM.</p> <p>The increase in cumulative growth was due to stimulation of the initial, linear growth phases.</p>	Dose dependent and complete inhibition of follicle and fiber growth at 100nM	Harmon and Nevins 1994
Ciprofloxacin (CPF) Human fibroblasts	<p>The effect of CPF on cell viability is time dependent:</p> <ol style="list-style-type: none"> 1. CPF was not cytotoxic at any concentration when the cells were incubated for 24 hours 2. low concentrations (0.0129 and 0.032 mM) of CPF increased the cell survival in all incubation periods tested. 	Decreased viability was observed at 0.129 and 0.194mM (48h of exposure), and 0.129M (72 h of exposure)	Gürbay <i>et al.</i> 2002

TABLE 2. Examples of dermatology-relevant chemicals displaying hormetic dose-response relationship in tumor cell lines

Chemicals and Tumor cell lines	Possible mechanisms	References
Endogenous agonists		
Epidermal Growth Factor (EGF) Ovarian, Colon, Epidermoid, Breast	A431 cells: 1. the dual effect (stimulation/inhibition) of EGF on its proliferation is associated to differential pattern of MAP kinase activities which may involve the action of specific phosphatase(s). 2. dependent on the quantity of occupied EGF-R: a critical and restricted number of sites is involved in EGF-growth stimulation 3. low-dose stimulation is mediated by a minority population of high-affinity EGF-Rs.	Chajry <i>et al.</i> 1995, 1996; Dong <i>et al.</i> 1991; Kawamoto <i>et al.</i> 1983
Estrogen Colon, Breast	Colon cancer cells: Physiological concentrations of estradiol acting via the classical ER may have a proliferative effect. When there are high luminal concentrations of estrogenic compounds, they may act on low affinity estrogen binding sites that mediate the growth-inhibitory effect.	Xu and Thomas 1995
Progesterone Ovarian	HOSE and Oca cells: stimulation by progesterone at low concentrations, marked inhibition at high concentrations, both blocked by specific progesterone antagonist, confirming the specificity of the hormonal action.	Syed <i>et al.</i> 2001
Phyto compounds		
Daidzein Breast	1. Isoflavones elicit a biphasic response in the DNA synthesis and cell proliferation of the ER of positive human breast cancer cells. 2. Effects of daidzein and biochanin A on these cells appeared to be associated with the expression of P53.	Ying <i>et al.</i> 2002
Genistein Colon, Breast, Oral	1. binds to the ER at estrogen binding site, the formed complex then interacts with the ERE1 thereby promoting the transcription of estrogen regulated genes 2. MCF-7 cells: cell proliferative effects were mediated through ER, while antiproliferative effect was independent of ER.	Miodini <i>et al.</i> 1999; Wang <i>et al.</i> 1996
Glabridin Breast	Proliferation of ER ⁺ cells was highly associated with the binding affinity of glabridin to the ER. Optimal cell proliferation occurred at a concentration at which 1/2 of the ER sites were saturated	Tamir <i>et al.</i> 2000

Table 2 continued on next page

TABLE 2. Examples of dermatology-relevant chemicals displaying hormetic dose-response relationship in tumor cell lines, *continued*

Chemicals and Tumor cell lines	Possible mechanisms	References
Quercetin Breast, Oral	<ol style="list-style-type: none"> 1. similar to Genistein, a biphasic effect on cell proliferation with ER involvement. 2. regulatory over-corrections by biosynthetic control mechanisms to low levels of growth inhibiting challenge 3. concentration-dependent antioxidant and prooxidant activities. 	van der Woude <i>et al.</i> 2003
Resveratrol Breast, Leukemia	<ol style="list-style-type: none"> 1. MCF-7 cells: at low concentration, acts as a partial ER agonist. At high concentrations, causes inhibition of MCF-7 cells regardless of ER status, possibly via the antagonizing of linoleic acid (a potent stimulator of breast cancer cells) 	Nakagawa <i>et al.</i> 2001
Drugs Dexamethasone Neuroepithelial, Pancreas, Meningiomas	<ol style="list-style-type: none"> 1. Brain tumor: Low-dose stimulation is related to the presence of glucocorticoid receptor (probably necessary but insufficient). Inhibitory effects at high doses was believed not to be due to receptor mediation but by other mechanisms such as cell membrane alterations. 2. Neuroepithelial cancer cells: <ol style="list-style-type: none"> a. dexamethasone treatment causes glucocorticoid receptors translocation into nucleus to modulate cell proliferation upon binding of different concentrations of dexamethasone. Dexamethasone inhibits proliferation of some neuroepithelial cell lines, not by glucocorticoid-induced apoptosis b. lower concentrations of dexamethasone stimulate growth only in glucocorticoid-positive tumors, suggested the role of the specific receptor. Higher concentrations inhibits cell growth not due to receptor mediation, but seems to be related to other mechanisms (cell membrane alterations) 	Gibelli <i>et al.</i> 1989; Kawamura <i>et al.</i> 1998; Paoletti <i>et al.</i> 1990
Retinoic acid Breast, Prostate, Glioblastoma	<ol style="list-style-type: none"> 1. Breast MCF-7 cells: via ICF-1 receptor: lowering ICF-1 levels inhibits cell proliferation. 2. Prostate LNCaP cells: possible roles of retinal-binding proteins and retinoic receptors which may have biphasic mitogenic effects on LNCaP cells and are concentration-dependent in affecting PSA secretion. 	Bentel <i>et al.</i> 1995; Fong <i>et al.</i> 1993

Table 2 continued on next page

TABLE 2. Examples of dermatology-relevant chemicals displaying hormetic dose-response relationship in tumor cell lines, *continued*

Chemicals and Tumor cell lines	Possible mechanisms	References
Toxic substances		
Cadmium chloride Ovarian	No cytotoxicity at low concentration, but has stimulatory effects on metabolic activities particularly in mitochondria via unknown mechanism	Abe <i>et al.</i> 1999
Sodium butyrate Colon	Dependent on the other energy sources available to epithelium: in conditions of low energy availability, butyrate could be both stimulatory/trophic. In the presence of high levels of alternative energy sources such as glucose, butyrate could inhibit growth/ induce apoptosis.	Singh <i>et al.</i> 1997

2004a; Rattan and Ali, 2007). While some important issues with respect to establishing the optimal hormetic conditions are yet to be resolved by future research, hormesis could be a promising approach for modulating aging (Rattan 2004b; Rattan 2004c).

DISCUSSION

Despite the acknowledgement of the existence of hormesis, its importance has been disregarded by the scientific community, with one of the reasons being the close association of the concept of hormesis with the highly controversial medical practice of homeopathy (Burgdorf and Happle 1996; Clement 1997; Cook and Calabrese 2006).

Calabrese and Blain (2005) developed a hormesis database, containing 5600 hormetic-like dose response relationships over approximately 900 agents from a broadly diversified spectrum of chemical classes and physical agents, stressing the general robustness of published studies to establish support for the hormetic dose-response hypothesis. Table 1 showed that clear examples of hormesis do exist in dermatology, and Table 2 suggested that the presence of hormesis in cancer biology may be an important phenomenon not to be overlooked.

Despite the extensive observation of hormetic dose-response relationships for numerous agents across the biological spectrum, most studies assessed cellular responses. Few studies followed up in animal and human models—normal or disease, or assessed the simultaneous responses of different systems to the same agent.

We believe *in vivo* studies are necessary to provide an integrative assessment of the whole animal/human responses to various agents, to document any discrepancies between the *in vitro* and *in vivo* responses, and to clarify the clinical implication of hormesis.

Studies on the mechanism of action and the exact definition of the low-dose to be applied are essential to achieve a better understanding of hormesis. Another important issue to discuss in the field of hormesis, as proposed by van der Woude *et al* (2005), is the need for risk assessment paradigms to be modified to take hormesis into account. Note that research methods may need to be modified to take into account that using lower doses and getting smaller responses may mean that a substantial increase in the number of animals or test subjects may be needed to get statistically significant results. Rietjens and Alink (2006) also suggested that more focus should be redirected from looking only at adverse effects at high levels of exposure to characterizing the complex biological effects, both adverse and beneficial, at low levels of exposure. Low-dose toxicology and pharmacology will contribute to better methods for low-dose risk assessment of chemical compounds and their effect on carcinogenesis, taking into consideration that the ultimate biological effect of a chemical may vary with its dose, the endpoint or target organ considered, cellular interactions, and/or the combined exposure with other chemicals.

We believe skin is an excellent candidate to gain entrance into this biology due to its accessibility; its complex nature, with highly differentiated cell types and various subsystems (keratinocytes, melanocytes, Langerhans' cells, fibroblasts, epidermis, dermis, hair follicle, eccrine, apocrine and sebaceous units); and the availability of specialized non-invasive technology for *in vivo* studies (Maibach 1996; Elsner *et al.* 2001). In addition, skin has been among the first organs analyzed using DNA microarrays in various topics from skin cancers, melanomas, basal cell carcinomas, squamous cell carcinomas, psoriasis and other inflammatory disorders, to stem cell biology, the biology of epidermal keratinocytes, and so forth (Table 3) (Blumenberg 2006). DNA microarray studies will be an excellent tool to elucidate the mechanisms of hormesis in skin biology. In short, better understanding of hormesis will likely lead to different strategies for risk assessment process employed in the fields of dermatologic toxicology and pharmacology.

CONCLUSION

Hormesis is a common phenomenon in dermatology and other fields. Detailed consideration should be given to its concept, its risk assessment implications, and its clinical significance. However, without additional mechanistic insight, the consequences of hormesis for risk assessment and the possibilities for *in vitro* to *in vivo* extrapolation will remain limited.

TABLE 3. Targets for DNA microarray studies in dermatology and skin biology (Blumenberg 2006)

Cell types
Keratinocyte differentiation
Melanoma and melanocytes
Epidermal stem cells and the hair cycle
Fibroblasts and other cutaneous cell types
Artificial skin substitutes
Study conditions
Wound healing and inflammatory diseases
Proinflammatory and immunomodulating cytokines in skin
Effects of UV and environmental stress
Carcinomas (basal cell carcinoma, squamous cell carcinoma)

Skin can be an excellent candidate to study hormesis and its underlying mechanisms because of its accessibility; its repertoire of inflammatory and immunomodulating cytokines, hormones, vitamins, unique responses to ultraviolet light, toxins, and physical injury; and the availability of non-invasive bioengineering and DNA microarray technology. Artificial skin substitutes are also available to study the effects of harmful or dangerous agents. In essence, the skin has everything: from stem cells, signaling and cellular differentiation, to inflammation, diseases, and cancer. All these facets could become excellent models to further study hormesis and its clinical implications following exposure to a variety of toxic compounds and pharmaceutical agents.

REFERENCES

- Abe T, Gotoh S, and Higashi K. 1999. Attenuation by glutathione of hsp72 gene expression induced by cadmium in cisplatin-resistant human ovarian cancer cells. *Biochem Pharmacol* 58: 69-76
- Bentel JM, Lebwohl DE, Cullen KJ, Rubin MS, Rosen N, Mendelsohn J, and Miller Jr. WH. 1995. Insulin-like growth factors modulate the growth inhibitory effects of retinoic acid on MCF-7 breast cancer cells. *J Cell Physiol* 165:212-221
- Bloom E, Sznitowska M, Polansky J, Ma ZD, and Maibach HI. 1994. Increased proliferation of skin cells by sublethal doses of sodium laurel sulfate. *Dermatology* 188:263-268
- Blumenberg M. 2006. DNA microarrays in dermatology and skin biology. *J Integrat Biol* 10(3): 243-257.
- Boyer N, Galey I, and Bernard BA. 1997. Biphasic effects of minoxidil on the proliferation and differentiation of normal human keratinocytes. *Skin Pharmacol* 10:206-220
- Burgdorf WH and Happle R. 1996. What every dermatologist should know about homeopathy. *Arch Dermatol* 955-958
- Calabrese EJ. 2001. Overcompensation stimulation: A mechanism for hormetic effects. *Crit Rev Toxicol* 31 (4&5): 425-470
- Calabrese EJ. 2005a. Hormetic dose-response relationships in immunology: Occurrence, quantitative features of the dose-response, mechanistic foundations and clinical implications. *Crit Rev Toxicol* 35 (6):89-295
- Calabrese EJ. 2005b. Cancer Biology and Hormesis: Human tumor cell lines commonly display hormetic (biphasic) dose responses. *Crit Rev Toxicol* 35 (6):463-582
- Calabrese EJ and Baldwin LA. 1997. The dose determines the stimulation (and poison): Development of a chemical hormesis database. *Int J Toxicol* 16:545-559

- Calabrese EJ and Baldwin LA. 2001. The frequency of U-shaped dose-responses in the toxicological literature. *Tox Sci* 62:330-338
- Calabrese EJ and Baldwin LA. 2003. The hormetic dose response model is more common than the threshold model in toxicology. *Tox Sci* 71(2):246-250
- Calabrese EJ and Blain R. 2005. The occurrence of hormetic dose responses in the toxicological literature, the hormesis database: an overview. *Toxicol Appl Pharmacol* 202: 289-301
- Chajry N, Martin PM, Pages G, Cochet C, Afdel K, and Berthois Y. 1995. Relationship between the MAP kinase activity and the dual effect of EGF on A431 cell proliferation. *Biochem Biophys Res Commun* 203:984-990
- Chajry N, Martin PM, Cochet C, and Berthois Y. 1996. Regulation of p42 mitogen-activated-protein kinase activity by protein phosphatase 2A under conditions of growth inhibition by epidermal growth factor in A431 cells. *Eur J Biochem* 235:97-102
- Clement RT. 1997. What every dermatologist should know about homeopathy, hormesis, and pharmacological inversion. *Arch Dermatol* 133: 245.
- Cook R and Calabrese EJ. 2006. The importance of hormesis to public health. *Environ Health Perspect* 114 (11): 1631- 1635
- Cotovio J, Roguet R, Pion FX, Rougier A, and Leclaire J. 1996. Effect of imidazole derivatives on cytochrome P-450 enzyme activities in a reconstructed human epidermis. *Skin Pharmacol* 9:242-249
- Dong XF, Berthois Y, and Martin PM. 1991. Effect of epidermal growth factor on the proliferation of human epithelial cancer cell lines: Correlation with the level of occupied EGF receptor. *Anticancer Res* 11:737-744
- Elsner P, Berardesca E, Wilhelm KP, and Maibach HI. 2001. *Bioengineering of the Skin*, CRC Press, Boca Raton, USA.
- Fong CJ, Sutkowski DM, Braun EJ, Bauer KD, Sherwood ER, Lee C, and Kozlowski JM. 1993. Effects of retinoic acid on the proliferation and secretory activity of androgen-responsive prostatic carcinoma cells. *J Urol* 149:1190-1194
- Gennings C, Carter Jr WH, Campain JA, Bae DS, and Yang RSH. 2002. Statistical analysis of interactive cytotoxicity in human epidermal keratinocytes following exposure to a mixture of four metals. *J Agric Biol Environ Stat* 7(1): 58-73
- Gibelli N, Zibera C, Butti G, Assietti R, Sica G, Scerrati M, Iacopino F, Roselli R, Paoletti P, della Cuna GR, and Rossi GF. 1989. Hormonal modulation of brain tumor growth: a cell culture study. *Acta Neurochir. (Wien)* 101:129-133
- Graham-Evans B, Tchounwou PB, and Cohly HHP. 2003. Cytotoxicity and proliferation studies with arsenic in established human cell lines: keratinocytes, melanocytes, dendritic cells, dermal fibroblasts, microvascular endothelial cells, monocytes and T-cells. *Int J Mol Sci* 4: 13-21
- Gürbay A, Garrel C, Osman M, Richard MJ, Favier A, and Hincal F. 2002. Cytotoxicity in ciprofloxacin-treated human fibroblast cells and protection by vitamin E. *Hum Exp Toxicol* 21:635-641
- Harmon CS and Nevins TD. 1994. Biphasic effect of 1,25-Dihydroxyvitamin D₃ on human hair follicle growth and hair fiber production in whole-organ cultures. *J Invest Dermatol* 103: 318-322
- Kawamoto T, Sato JD, Le A, Polikoff J, Sato GH and Mendelsohn J. 1983. Growth stimulation of A431 cells by epidermal growth factor: Identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. *Proc Natl Acad Sci USA* 80:1337-1341
- Kawamura A, Tamaki M, and Kokunai T. 1998. Effect of dexamethasone on cell proliferation of neuroepithelial tumor cell lines. *Neurol. Med. Chir. (Tokyo)* 38:633-640
- Krischel V, Bruch-Gerharz D, Suschek C, Kroncke KD, Ruzicka T, and Kolb-Bachofen V. 1998. Biphasic effect of exogenous nitric oxide on proliferation and differentiation in skin derived keratinocytes but not fibroblasts. *J Invest Dermatol* 111: 286-291
- Maibach HI. 1996. *Dermatologic Research Techniques*, CRC Press, Boca Raton USA
- Miodini P, Fioravanti L, Di Fronzo G, and Capelletti V. 1999. The two phyto-oestrogens genistein and quercetin exert different effects on oestrogen receptor function. *Br J Cancer* 80:1150-1155
- Nakagawa H, Kiyozuka Y, Uemura Y, Senzaki H, Shikata N, Hioki K, and Tsubura A. 2001. Resveratrol inhibits human breast cancer cell growth and may mitigate the effect of linoleic acid, a potent breast cancer cell stimulator. *J Cancer Res Clin Oncol* 127:258-264
- Paoletti P, Butti G, Zibera C, Scerrati M, Gibelli N, Roselli R, Magrassi L, Sica G, Rossi G, and della Cuna GR. 1990. Characteristics and biological role of steroid hormone receptors in neuroepithelial tumors. *J Neurosurg* 73:736-742

- Rattan SI. 2004a. Mechanisms of hormesis through mild heat stress on human cells. *Ann N Y Acad Sci* 1019:554-558
- Rattan SI. 2004b. Aging intervention, prevention, and therapy through hormesis. *J Gerontol A Biol Sci Med Sci* 59(7):705-709
- Rattan SI. 2004c. Hormetic mechanisms of anti-aging and rejuvenating effects of repeated mild heat stress on human fibroblasts in vitro. *Rejuvenation Res* 7(1):40-48
- Rattan SI and Ali RE. 2007. Hormetic prevention of molecular damage during cellular aging of human skin fibroblasts and keratinocytes. *Ann N Y Acad Sci* 1100:424-430
- Rietjens IMCM and Alink GM. 2006. Future of Toxicology - low-dose toxicology and risk-benefit analysis. *Chem Res Toxicol* 19(8): 977-981
- Singh B, Halestrap AP, and Paraskeva C. 1997. Butyrate can act as a stimulator of growth or inducer of apoptosis in human colonic epithelial cell lines depending on the presence of alternative energy sources. *Carcinogenesis* 18:1265-1270
- Smith BP, Gale KL, Einspinner M, Greenstock CL, and Gentner NE. 1992. Stimulated human fibroblast cell survival/clonogenicity in response to low doses of ionizing radiation. In: Sugahara T, Sagan LA, and Aoyama T (eds), *Low dose irradiation and biological defense mechanisms*, pp. 157-164 Elsevier Science Publishers B.V., Amsterdam, The Netherlands
- Syed V, Ulinski G, Mok SC, Yiu GK, and Ho SM. 2001. Expression of gonadotropin receptor and growth responses to key reproductive hormones in normal and malignant human ovarian surface epithelial cells. *Cancer Res* 61:6768-6776
- Tamir S, Eizenberg M, Somjen D, Stern N, Shelach R, Kaye A, and Vaya J. 2000. Estrogenic and antiproliferative properties of glabridin from licorice in human breast cancer cells. *Cancer Res* 60:5704-5709
- van der Woude H, Alink GM, and Rietjens IMCM. 2005. The definition of hormesis and its implications for in vitro to in vivo extrapolation and risk assessment. *Crit Rev Toxicol* 35:603-607
- van der Woude H, Gliszczynska-Swiglo A, Struijs K, Smeets A, Alink GM, and Rietjens IMCM. 2003. Biphasic modulation of cell proliferation by quercetin at concentrations physiologically relevant in humans. *Cancer Lett* 200(1): 41-47
- Varani J, Astrom A, Griffiths CEM, and Voorheers JJ. 1991. Induction of proliferation of growth-inhibited keratinocytes and fibroblasts in monolayer culture by sodium lauryl sulfate: comparison with all-trans retinoic acid. *J Invest Dermatol* 97:917-921
- Vega L, Styblo M, Patterson R, Cullen W, Want C, and Germolec D. 2001. Differential effects of trivalent and pentavalent arsenicals on cell proliferation and cytokine secretion in normal human epidermal keratinocytes. *Toxicol Appl Pharmacol* 172:225-232
- Wang TTY, Sathyamoorthy N, and Phang JM. 1996. Molecular effects of genistein on estrogen receptor mediated pathways. *Carcinogenesis* 17:271-275
- Xu X and Thomas ML. 1995. Biphasic actions of estrogen on colon cancer cell growth: Possible mediation by high- and low-affinity estrogen binding sites. *Endocrine* 3: 661-665
- Ying C, Hsu JT, Hung HC, Lin DH, Chen LFO, and Wang LK. 2002. Growth and cell cycle regulation by isoflavones in human breast carcinoma cells. *Reprod Nutr Dev* 42:55-64
- Yokoo S, Furumoto K, Hiyama E, and Miwa N. 2004. Slow-down of age-dependent telomere shortening is executed in human skin keratinocytes by hormesis-like-effects of trace hydrogen peroxide or by anti-oxidative effects of pro-vitamin C in common concurrently with reduction of intracellular oxidative stress. *J Cell Biochem* 93:588-597
- Zhang TC, Schmitt MT, and Momford JL. 2003. Effects of arsenite on telomerase and telomeres in relation to cell proliferation and apoptosis in human keratinocytes and leukemia cells in vitro. *Carcinogenesis* 24(11):1811-1817
- Zouboulis CC, Seltmann H, Hiroi N, Chen WC, Young M, Oeff M, Scherbaum WA, Orfanos CE, McCann SM, and Bornstein SR. 2002. Corticotropin-releasing hormone: An autocrine hormone that promotes lipogenesis in human sebocytes. *Proc Natl Acad Sci USA* 99(10): 7148-7153