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COMMENTARY

Role of Phenytoin in Wound Healing–A Wound Pharmacology Perspective

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ABSTRACT. Topical agents used for the enhancement of wound healing are designed to act locally and, therefore, do not undergo classic systemic metabolic modification. This commentary reviews the potential role of a vulnerary agent, phenytoin (PHT), from a wound pharmacology perspective. This agent may have the potential to alter the dynamics of wound healing, suggesting a therapeutic use for the stimulation of chronic wounds. Oral PHT therapy is used widely for the treatment of convulsive disorders, and about half the patients treated develop gingival overgrowth as a side-effect. This apparent stimulatory effect has prompted its assessment in wound healing. Investigations into the mechanisms of gingival overgrowth also provide clues to its action in wound healing, and important similarities and differences are discussed. It appears also that both gingiva and skin are important extrahepatic sites for xenobiotic metabolism, and analysis of the biochemical mechanisms should lead to the design of safer analogues for wound healing. On the other hand, differences between the pharmacokinetics of topical PHT in these tissue situations indicate that different formulations are required for gingival and cutaneous wound healing and during the changing course of wound healing itself. BIOCHEM PHARMACOL **57**;10:1085–1094, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. phenytoin; wound healing; gingival hyperplasia; cytochromes P450

RECENT TRENDS IN WOUND HEALING

Many different types of trauma result in skin injury. The body is usually capable of closing these wounds spontaneously to restore its protective function as a barrier and maintain homeostasis. In lower vertebrates, complete tissue regeneration can occur, whereas in higher vertebrates the loss of normal architecture results in a fibroproliferative response, producing a scar. Delay in healing may occur with large wounds or impaired tissue viability and results in enhanced scarring. "Healing" is often considered as complete when a wound is epithelialised, although cellular events continue for months or years. Non-healing or chronic wounds take a great toll on diabetic and venous insufficiency patients in terms of morbidity and mortality, while incurring great health-care expenditure.

The quest for wound healing agents is perhaps one of the oldest challenges for medical practice, and various treatments have long been recorded in ancient writings [1]. In the late 20th century, we have moved from an era of solely removing noxious influences (e.g. infection, inflammation, necrotic tissue) and pain control, to one in which we visualise the healing process being positively fostered. Recently, much attention has been focused on early foetal wound healing, which is so nearly perfect as to be considered scarless [2]. Manipulation of adult wounds with the aim of producing "scarless" healing and full regeneration is currently a Utopia, which may never be reached. We still need a deeper understanding of tissue repair mechanisms in order to intervene at cellular or molecular levels. The best we can hope for is to pharmacologically reduce the time to heal, the cost of care in certain cases, and to modify to some extent the end result of function and cosmesis.

Since the discovery of the first growth factor, it has been shown that cellular events in wounds could be potentially accelerated. Such agents in experimental trials are usually delivered topically and, therefore, have different pharmacodynamics and kinetics from conventional, orally administered drugs. Unlike topical agents administered through transdermal devices, vulnerary agents are intended to localise and exert their effect in the wound only. Therefore, serum concentrations will not reflect their efficacy, but only indicate possible toxicity if systemic absorption were to take place. The need to establish this new discipline of wound pharmacology was recognised by the European Tissue Repair Society in 1995 [3], which encouraged new criteria for the evaluation of the safety and efficacy of topically applied wound healing agents. These require careful choices of suitable experimental systems and physiologically relevant bioassays.

It is the aim of this commentary to examine one such

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vulnerary agent, PHT*, from a wound pharmacology perspective. A common side-effect with prolonged PHT treatment for epilepsy is the development of fibrous overgrowth of the gingivae [4], although mild skin and skull thickening may also occur. This apparent stimulatory effect on connective tissue suggests an exciting possibility for use in wound healing. Despite numerous clinical trials and case studies carried out world-wide, the mechanism of action of PHT in promoting wound healing is still unknown.

CLINICAL PHARMACOLOGY OF ORAL PHT

PHT is related to the barbiturates in chemical structure (but has a five-membered ring), though it does not cause sedation and is non-habit forming. Its primary site of action is in the motor cortex, where spread of seizure activity is inhibited by a membrane potential-dependent blockade of Na^+ channels and perhaps presynaptic Ca^{2+} channels [5]. Oral therapy is usually initiated with 300 mg/day, and steady-state therapeutic levels are achieved after 7–10 days. The clinically effective serum level for epilepsy is usually between 10 and 20 μg/mL, and levels higher than 20 μg/mL induce dose-related side-effects [6]. The pharmacokinetics of oral PHT are well documented [7].

PHT is metabolised in the liver (Fig. 1) by a saturable cytochrome P450 enzyme system. A study by Doecker *et al.* [10] identified the role of the CYP2C3 isozyme in the hydroxylation process. The first step of these metabolic pathways, however, is the formation of an unstable intermediate, or "arene oxide," which may covalently bind to target tissues. Such compounds are potentially carcinogenic, mutagenic, and have hepatotoxic effects.

Because PHT is an asymmetric molecule, the carbon-5 of the hydantoin ring represents a prochiral centre (Fig. 2). The ratio of diastereoisomers of p-HPPH and DHD in humans is 3(*S*):1(*R*) [11]. It is not known whether the separate diastereoisomers are the product of separate isozymes or if the enzyme lacks total substrate specificity. Ieiri and co-workers [11] have shown recently that patients with PHT-induced gingival overgrowth have an unusually high level of the (*R*)- rather than the (*S*)-enantiomer of p-HPPH. (*R*)-p-HPPH, the least abundant metabolite, was also responsible for increasing gingival fibroblast proliferation *in vitro*. This pioneering investigation was the first study to compare the pharmacologic properties of separate enantiomers; all other studies used racemates of p-HPPH. Interestingly, reduced (*R*)-enantiomer production also can be correlated with poor mephenytoin metabolism. As wide interethnic mephenytoin hydroxylation differences are documented, Ieiri and co-workers have also suggested that

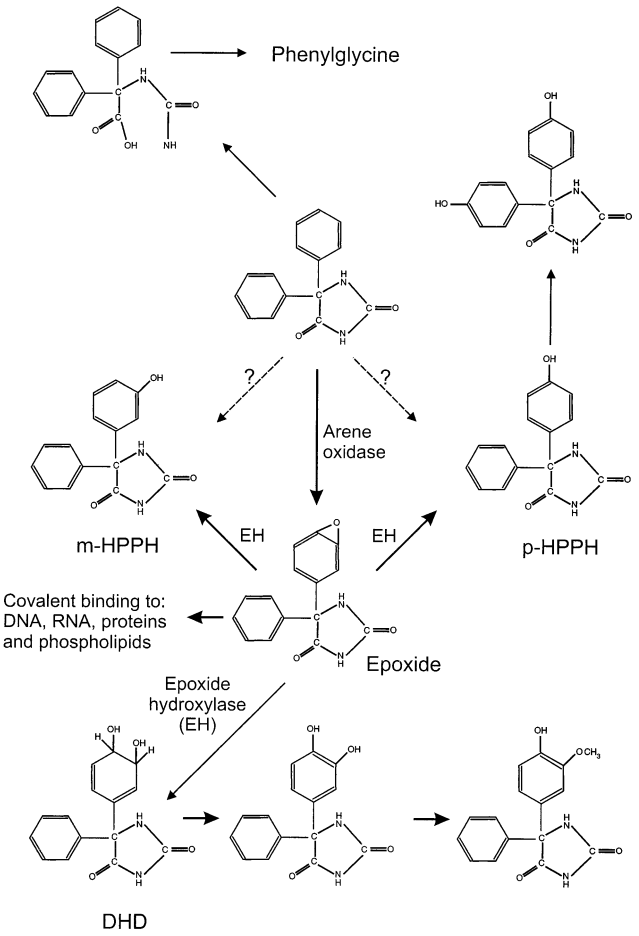

FIG. 1. Summary of the oxidative metabolism of PHT in humans. The major metabolic routes involve the formation of 5-(4-hydroxyphenyl)-5-phenylhydantoin (p-HPPH), 5-(3,4-dihydroxy-1,5-cyclohexadiene)-5-phenylhydantoin (DHD), and smaller amounts of 5-(3-hydroxyphenyl)-5-phenylhydantoin (m-HPPH) [8, 9].

considerable variations in PIGO may occur among populations of different origin. Based on this theory, Caucasians are the most prone to PIGO and East Asians the least.

PHT-INDUCED GINGIVAL PATHOLOGY

PHT-Induced Gingival Overgrowth

Approximately half the patients treated with PHT develop gingival overgrowth (Fig. 3). Considerable controversy persists as to whether it should be classed as a hypertrophy, hyperplasia, or fibrosis. An extensive histopathological study by Hassell *et al.* [12] claimed that it merely represents uncontrolled growth with normal connective tissue composition. This implies that the lesion contains an increased number of cells, which, in turn, produce excessive gingival tissue. Histologically (Fig. 4), the lesion is characterised by epithelial acanthosis, but the main feature is the excessive accumulation of collagen fibre bundles in the dermis. Dill and Iacopino [13] also observed the presence of myofibroblasts in human PIGO, which may play a role in its pathogenesis. Myofibroblasts are thought to be involved in

* *Abbreviations:* PHT, phenytoin (5,5-diphenylhydantoin or 5,5-diphenyl-2,4-imidazolidinedione; Phenytoin, Dilantin, Epanutin); p-HPPH, *p*-hydroxyphenyl-5-phenylhydantoin or 5-(4-hydroxyphenyl)-5-phenylhydantoin; PIGO, phenytoin-induced gingival overgrowth; m-HPPH, *m*-hydroxyphenyl-5-phenylhydantoin or 5-(3-hydroxyphenyl)-5-phenylhydantoin; and DHD, dihydrodiol or 5-(3,4-dihydroxy-1,5-cyclohexadiene)-5-phenylhydantoin.

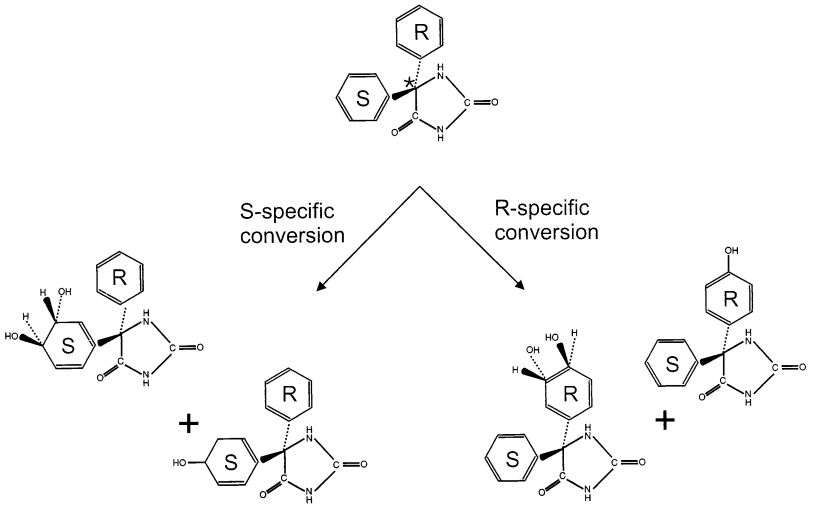

FIG. 2. Stereoisomeric configurations of the major PHT metabolites (p-HPPH and DHD).

the pathogenesis of certain fibrotic diseases such as hypertrophic scars and Dupuytren's disease, but not keloids [14].

Gingival Metabolism of PHT—Local Metabolism

Although there are many proposed mechanisms put forward for PIGO, few theories explain why the gingiva is principally involved. Periodontium and healing wounds have been suggested to be the most dynamic and metabolically active tissues in the entire human body [15, 16], perhaps one reason why the effects of PHT are most prominent in these. Moreover, after oral administration, the bioavailability of PHT is greater in gingival tissue than in normal skin or bone, hence the difference in the severity of the side-effects at these locations [7].

Measurements of PHT and its major metabolite in serum, gingiva, and saliva suggested local metabolism in humans [17]. It is postulated that gingival tissues are exposed to PHT from both blood and saliva, and, additionally, plaque may act as a reservoir for the drug. Therefore, both systemic and reabsorbed PHT in combination maintain unbound drug (or metabolite levels), which after prolonged exposure may cause PIGO.

Steinberg [18], investigating the fate of topically applied [^{14}C]PHT in rabbit models, found that the gingival sulcus had a great capacity for the uptake of PHT. After 2 hr, most of the radioactivity was found to be in the cerebral cortex and the least in serum. However, gingiva appeared to retain the label for the longest time. As metabolic conversion is thought to be essential for covalent binding [19], this has instigated further research into the metabolic capacity of the oral mucosa. A breakthrough study by Zhou *et al.* in 1996 [20] revealed that CYPs were present here in concentrations as much as 50-fold greater than in hepatic microsomes, even though the number of microsomes found in oral mucosa was only about one-tenth that in liver. The activity of CYP1A1, CYP1A2, CYP2E1, CYP3A4, and CYP2C9 was also measured, but no CYP2B6 or CYP2D6

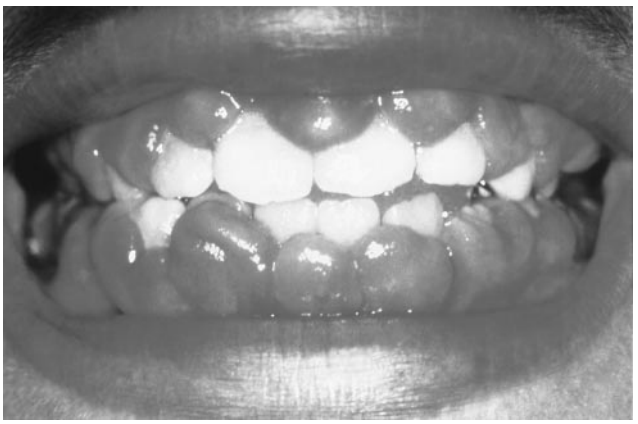

FIG. 3. Patient with PHT-induced gingival overgrowth (courtesy of Prof. P. Speight, Eastman Dental Institute; slide reproduced with permission). Note the enlarging interdental papillae between central and lateral incisors. The lesion is age and site specific. It occurs mainly within the gingiva propria of young individuals and is rarely seen in persons above 35–40 years of age.

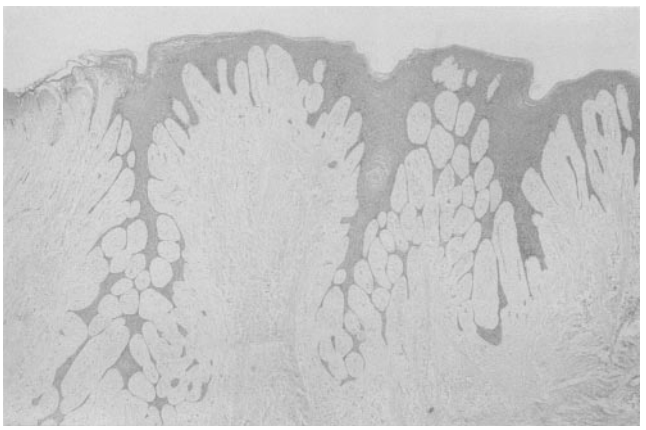

FIG. 4. High power view of H&E section through PHT-induced gingival lesion (courtesy of Prof. P. Speight, Eastman Dental Institute; slide reproduced with permission). The lesion is characterised by epithelial acanthosis, with long, branched rete peg formation, but the main feature is the excessive accumulation of radiating collagen fibres in the dermis.

was detected. Microsomes obtained from gingiva showed significant PHT hydroxylase activity as determined by the production of p-HPPH (12.8 to 276.9 pmol HPPH/min·mg microsomal protein).

As cytochrome P450-dependent monooxygenase [21] and epoxide hydrolase [22] are also documented in human skin, it appears that gingiva and skin are important extrahepatic sites for xenobiotic metabolism. Unlike skin, however, oral mucosa is not as heavily cornified, and the absorption of ingested agents is not so limited. This was confirmed *in vitro* when the penetration of tritiated water and tritiated red tide toxin was compared in monkey buccal mucosa and skin [23].

Putative Mechanisms of Action of PHT in the Pathogenesis of Gingival Overgrowth

The enigma of PIGO has occupied researchers for decades. Therefore, considerably more information is available on this topic than on the role of PHT in wound healing. The subject of drug-induced hyperplasia has been excellently reviewed by Brown *et al.* [24], and its detailed discussion is beyond the scope of this commentary. These studies, however, may give clues as to how PHT may up-regulate connective tissue activity in wound healing, although some routes are only relevant to gingiva. Briefly, the following factors have been suggested to play key roles in its induction, based on this review [24] together with recent advances:

- PHT pharmacokinetics and tissue binding
- PHT local metabolism and metabolites [11, 20]
- Inflammation from bacterial plaque
- Elevated number of Langerhans cells in gingiva [25]
- Immunoglobulin induction by PHT
- p-HPPH potentiation of interleukin-1-induced prostaglandin biosynthesis by gingival fibroblasts [26]
- Gingival fibroblast phenotype changes
- Presence of myofibroblasts [13]
- Perturbed collagen metabolism
- Disruption of fibroblast Na^+/Ca^{2+} flux, increase in intracellular calcium in gingival fibroblasts
- Epidermal growth factor (EGF) receptor up-regulation
- 5α-Dihydrotestosterone receptor up-regulation
- Inhibition of folic acid uptake
- Combination theories (involving several of the above)

In certain ways, the study of the effects of PHT on connective tissue has proven to be difficult. Contradictory results have been documented, according to which PHT may either increase or decrease synthetic and proliferative activity or have no effect at all *in vitro*. These inconsistencies are due to the fact that the drug effects are reportedly dependent on cell type (sources of fibroblasts), cell density, phase of the cell cycle, and drug concentrations [27]. Furthermore, both Hassell and Gilbert [28] and Benveniste and Bitar [27] have demonstrated that within gingival tissue a PHT-sensitive, genetically determined [29] subpopulation exists, which may dominate *in vivo* treatment outcomes. A study of hyperplasia with dermal fibroblasts [30] claimed that such a reaction can be either monoclonal or oligoclonal in origin. These so-called "responder cells" retain their features through several passages *in vitro*, in the absence of the drug. Therefore, *in vitro* studies using previously untreated cells may only be useful to indicate that clonal selection is a likelihood.

Fibroblast heterogeneity in skin and wounds is also well documented [15]. These subpopulations differ in their morphology, cell surface markers, proliferation rate, collagen synthesis, and growth factor production and response. Therefore, there is also potential for a different response to exogenously added substances. It is then vital to obtain a representative cell population to test the true nature of drug effects *in vitro*. Most studies use explants (as opposed to collagenase digestion) to obtain cells, thereby limiting diversity to the most migratory ones. A recent study by Eastwood *et al.* [31] has shown significant differences in contraction of dermal fibroblasts obtained from the same patient using these two methods.

The *in vivo* study of the cause of PIGO has been obscured by the fact that PHT-induced pathogenesis is species-specific, and only a limited number of animal models are available. Also, not all animals metabolise PHT in the same way. Perhaps the production of reactive metabolites and the presence of responder cells combined hold the key to the understanding of the mechanism of action of PHT on connective tissue.

PHT AND PERIODONTAL HEALING

The first controlled clinical trial of wound healing was carried out by Shapiro in 1958 [32] treating periodontal patients with oral PHT prior to surgery, resulting in accelerated healing of their gingiva and reduced inflammation and pain compared with control individuals. It was Shapiro who initially suggested that PHT may also be useful to increase the rate of wound healing in other areas of the body. Since then, further studies confirmed its beneficial effects, when PHT was incorporated into gels and pastes to be applied topically for the treatment of periodontal diseases [33] and for the promotion of healing of dental extraction sockets [34]. In the 1970s, a commercial topical PHT preparation was available from Laboratoires Roussel, France, for the treatment of periodontal diseases [33]. A decade later, attempts have been made to evaluate the efficacy of a p-HPPH analogue in human gingival wound healing, and *p*-chlorophenytoin (as 1% dentifrice) was shown to accelerate healing rate twice as fast as PHT [35].

CLINICAL TRIALS WITH PHT IN CUTANOUS WOUND HEALING

The conduction of double-blind, randomised, placebo-controlled clinical trials with PHT presented many practical problems. The lack of suitable inert powder meant that the studies were neither controlled nor carried out in a double-blind fashion. Many controlled studies, on the other hand, used agents that influenced the wound healing process and, therefore, cannot be regarded as true controls. Because of these difficulties the number of non-randomised, retrospective trials or anecdotal accounts is vast. However, the outcomes can still be compared with historical controls, and a sense of efficacy could still be appreciated.

Favourable results were reported with topical PHT in the treatment of trophic ulceration in leprosy [36–39], venous stasis ulcers [40], decubitus ulcers [41–43], diabetic foot ulcers [44], ulcers of various aetiologies [41, 45], large abscess cavities [46, 47], burns [48–50], clean surgical wounds (split-thickness skin autograft donor sites) [51], and epidermolysis bullosa [52].

Advantages of Topical PHT Therapy

For the treatment of localised injuries, topical application gives direct access to the target site without undergoing classic metabolic pathways, and there is a lower risk of causing dose-related side-effects seen with systemic therapy for wound healing [53]. Nevertheless, oral PHT has been tested in diseases that can involve the entire integument (epidermolysis bullosa, lichen planus, discoid lupus erythematosus) with variable success. These open trials should be interpreted with caution, as these conditions undergo variable disease progression, and it is possible that the levels of PHT reaching the skin are below therapeutic levels for wound healing. For example, favourable responses were reported recently by Masgrau-Peya *et al.* [52] using a topical PHT cream in the treatment of epidermolysis bullosa. This represents a distinct approach, as it attempts to alleviate symptoms of a genetic disease rather than trying to resolve the underlying problem with systemic therapy [54], originally thought to be abnormally high collagenolytic activity.

In vivo and clinical studies with topical PHT claim:

- Acceleration in healing and granulation tissue formation [36–51]
- Reduction in oedema and inflammation, wound transudate and exudate [37, 41, 42, 46, 47, 49]
- Decrease of the bacterial load of wounds, therefore the need for antibiotic therapy [41, 42, 44–46]
- Possible facilitation of nerve regeneration [41]
- Provision of rapid pain relief [40, 42, 48–51]
- High success rate in difficult, chronic cases unresponsive to traditional therapies
- Safety: no adverse reactions were reported
- Low cost and availability as opposed to expensive alternatives, such as a mixture of synthetic growth factors [55] currently being evaluated

WOUND PHARMACOLOGY OF PHT

Surprisingly, most of the clinical trials with topical PHT were based on the daily application of a uniform layer of PHT powder of unknown quantity. Only Lodha *et al.* [46] specified the dose to be 20 mg/cm^2, which was sufficient to promote wound healing, but it could not be taken to be optimal. Clearly, dose-response studies are needed with topical PHT in the various wounds aetiologies.

It has been concluded (although few investigators have measured serum levels) that systemic absorption is not significant [43, 49, 52, 56]. A very recent report is perhaps the most striking of all case studies. Anstead *et al.* [43] treated an obese man with a massive sacrolumbar pressure ulcer requiring 12.5 g/day of bulk-grade PHT (several times the lethal daily oral dose) as a slurry in NaCl (0.9%) to cover it. Despite these large doses, 31 days after therapy commenced, the serum concentrations were only 4.3 mg/L. This is the only account that measured appreciable serum concentrations; however, this was also the largest wound ever to be treated with topical PHT.

The amount of topical agent being absorbed and entering the systemic circulation is dependent upon many factors [3]. Intact skin is an active barrier, particularly the dead cells of the stratum corneum, for many substances are impermeable, requiring the aid of penetration enhancers. Similarly, any eschar present in the wound will prove to be a barrier and needs to be debrided. If the target site for delivery is the basal layer of the epidermis and the dermis, re-epithelialisation will increasingly impede drug delivery with time, even though a fully differentiated stratum corneum is not yet present [57]. Therefore, a suitable method of delivery is required to ensure that adequate amounts of PHT reach the dermis. Alternatively, PHT may be used to stimulate epidermal activity if it is incorporated in low concentrations into an appropriate vehicle, as keratinocytes are more sensitive to it.* Other factors influencing its absorption are: the inhomogeneity of the wound, the presence of granulation tissue, tissue oedema, vasculature and perfusion, type of dressings, vehicle effects on skin permeability, release rate from the delivery system, and local drug interactions.

Referring to unpublished data, Modaghegh *et al.* [41] claimed that PHT powder gave the most favourable results in a rat experimental model testing four formulations (gel, cream, PHT sodium powder, and PHT powder). PHT is relatively lipid-soluble (log octanol/water partition coefficient = 2.23), it is likely that the solubility of powdered PHT in wound fluid (pH 7.07 to 7.2) is low, similar to that of plasma (pH 7.4, 75 μg/mL), and reasonable amounts will be either washed out with any exudate or lost on the dressing. Moreover, the presence of bacteria will lower the pH, decreasing its solubility. It is not known, however, if bacteria are capable of metabolising PHT.

Formulations for the enhancement of gingival wound healing used low concentrations (1%) of PHT in gels in

* Talas G and Brown RA, Manuscript submitted for publication.

line with the fact that systemic uptake from oral mucosa is a likelihood. Contrary to this, copious amounts of PHT powder were applied to chronic wounds where the entire epithelium was absent, without significant systemic absorption. One reason for this could be, apart from the low water solubility of PHT, that vascularity and perfusion are compromised in non-healing wounds.

Little is known about the distribution and metabolism of PHT within the wound. Given the nature of PHT and poor circulation in chronic wounds, it is feasible that PHT is retained there for longer periods and could be metabolised by cytochrome P450 isozymes present in the epidermis, sebaceous glands, the outer root sheath of the hair follicles, and the dermis [58]. Future, meaningful *in vitro* studies into the absorption of PHT in wounds should consider both diffusion and cutaneous biotransformation. Experimental models should also take into account that well known differences exist in enzyme activities and distribution between species, anatomical sites, and tissues. Considerable similarity exists concerning skin permeability properties for topical agents between human skin and species like miniature pigs, monkeys, and dogs, in that order. However, no such ranking has been established for the metabolic activity in skin.

PUTATIVE MECHANISMS OF ACTION OF PHT IN WOUND HEALING

Wound healing is a complex process involving the collaboration of heterogeneous groups of cells. During this highly organised sequence of events, keratinocytes, fibroblasts, endothelial and inflammatory cells communicate with each other via cytokines and other soluble mediators, but in some cases cell-cell contacts and cell-matrix interactions also act as regulatory factors. The subject of wound healing has been covered excellently in detail recently by Clark [59], and readers are encouraged to refer to it for supplementary reading and exhaustive references.

After injury, a temporary repair and protection are achieved by the formation of a blood clot, which is mainly composed of fibrin, aggregated platelets, blood cells, and, to a lesser extent, fibronectin and vitronectin. The substances released from the tissue debris provoke a classic, characteristic inflammatory reaction. In response to this, inflammatory cells (neutrophilic granulocytes, macrophages) attach to the scaffold provided by the thrombus and migrate through. These cells absorb and enzymatically degrade foreign materials, thereby cleaning the wound site, and release further soluble factors. The clot also serves as a reservoir of growth factors and cytokines, which not only attract inflammatory cells to the injured site, but also influence the latter stages of wound healing. Topical PHT was reported to reduce oedema and inflammation, wound transudate and exudate [37, 41, 42, 46, 47, 49], and speed up granulation tissue formation. Moreover, PHT decreased the bacterial load of wounds, therefore the need for antibiotic therapy. It was effective against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas* spp. (coagulase positive) in clinical studies within 7–10 days [41, 42, 44, 45]. In a guinea pig model of wound healing [46], PHT treatment led to enhanced clearance of gram-negative as opposed to gram-positive bacteria. It is as yet unknown whether PHT has an intrinsic bactericidal activity or if it has an indirect effect by affecting inflammatory cells and neovascularisation. PIGO interestingly contained an increased number of Langerhans cells, important in antigen gathering and presentation in the epithelial layers [25].

About 4 days after injury, new stroma begins to replace the thrombus. This granulation tissue formation entails connective tissue deposition and angiogenesis. Macrophages, fibroblasts, and blood vessels move into the wound space simultaneously. Macrophages provide the cytokines necessary for fibroplasia and angiogenesis, while fibroblasts deposit the new extracellular matrix supporting cell ingrowth. New blood vessels, on the other hand, deliver oxygen and nutrients for the sustenance of continued repair.

Biopsies of PHT-treated open wounds showed earlier appearance of mononuclear cells, eosinophils, fibroblasts, collagen deposition, neovascularisation, re-epithelialisation, and decreased polymorphonuclear exudate, indicating that its influence on wound healing could be by inducing cell proliferation and the recruitment of cells to the wound area. Although a modest increase in dermal fibroblast growth was observed *in vitro* with low concentrations of PHT [60, 61], it is unlikely that profound mitotic activity caused by the direct action of the drug is responsible for increased granulation tissue formation. PHT, however, may indirectly affect the proliferative, synthetic, and migratory activity of fibroblasts via growth factor expression, such as up-regulating platelet-derived growth factor (PDGF) release from macrophages and monocytes [62]. Nevertheless, PHT was found to exert a direct effect on fibroblasts as it was shown to be a more potent chemoattractant than fibronectin *in vitro*.* As both PDGF and its receptor expression are reduced and fibronectin is extensively degraded during impaired wound healing, topical PHT may aid chronic wound healing via these suggested routes. Checkerboard analysis revealed that PHT stimulated both chemotaxis and chemokinesis, suggesting its importance both in the initiation and directional maintenance of fibroblast migration.

To facilitate the movement through the cross-linked fibrin clot and a tight meshwork of extracellular matrix, a variety of fibroblast- and plasma-derived enzymes cleave the path for cell migration. PHT was also found to up-regulate one such agent, urokinase-type plasminogen activator expression in dermal fibroblasts [63].

Once the recruited fibroblasts reach the wound, they gradually become secretory. Their major function becomes protein synthesis (principally collagen), and this fibrotic phenotype predominates. A loose matrix of fibronectin is

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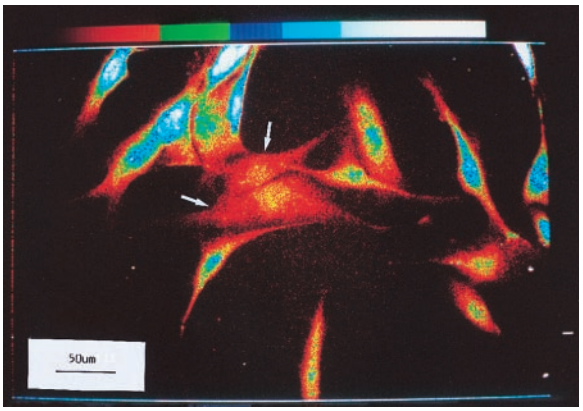

FIG. 5. Differential uptake, metabolism, and binding of PHT by human dermal fibroblasts. Cells were incubated with PHT (20 μg/mL) for 24 hr and stained for PHT by indirect immunofluorescence. Fluorescein isothiocyanate (FITC)-fluorescence was detected between 510 and 540 nm using a CCD camera (600 × 400 pixels; 1 μm resolution per pixel). The fluorescence was imaged quantitatively, and the signal was processed by an IBM personal computer into a falsely colour-coded microscopic image of the cells depicting the mean counts per pixel. Covalent binding occurred around the nucleus (white/blue areas), but not all fibroblasts were capable of metabolic conversion to the same extent. Fibroblasts indicated with the white arrow contained the least amounts of PHT.

produced, which serves as a template for collagen fibrillogenesis. Initially, types III, I, and V collagen are laid down to provide some tensile strength. Collagen turnover is a highly balanced process involving the healthy maintenance of its synthesis and degradation by fibroblasts, cytokines, matrix metalloproteinases and their inhibitors. The final deposition of proteoglycans and elastin also ensures resilience to deformation and elasticity. Currently, no information is available about the effects of topical PHT therapy on the composition of connective tissue of the wound or its tensile strength. Studies of PIGO, however, indicate elevated type III collagen and proteoglycan, and less type I collagen production, while fibronectin and type V collagen are not affected [24, 64]. PHT was claimed to have an indirect inhibitory effect on collagenase activity *in vitro* in both gingival [24] and dermal fibroblasts [60, 61], suppressing matrix degradation *in vivo*. It is also possible that, similarly to PIGO, clonal selection takes place in wounds, whereby PHT either recruits cells that have elevated migratory and secretory capabilities or may cause a phenotypic change in resident cells in the woundbed. This heterogeneity is reflected in the fact that not all dermal fibroblasts take up, metabolise, and bind PHT to the same extent (Fig. 5).

Angiogenesis occurs concurrently with fibroplasia. Although accelerated neovascularisation was seen in PHT-treated wounds, no information is available on its effect on endothelial cells. As vascularisation is also thought to be interrelated to nerve regeneration [65], perhaps this may explain an interesting case in which PHT facilitated nerve regeneration [41]. Moreover, topical PHT provides rapid pain relief [40, 42, 48–51], possibly by achieving local neuronal membrane stabilisation [66], although the uses of systemic PHT have also been reported in the management of various types of pain [67].

Re-epithelialisation begins within 24–48 hr after injury, and a sheet of epithelium gradually covers the moist vascular granulation bed. PHT was shown to modestly increase keratinocyte proliferation at low concentrations; however, higher concentrations were not as well tolerated as by fibroblasts.* *In vivo*, however, PIGO is characterised by acanthosis, indicative of elevated keratinocyte proliferation or migration. PHT was also found to be a potent chemoattractant *in vitro* for epithelial cells,* further supporting the idea that PHT promotes re-epithelialisation by enhancing keratinocyte motility.

Finally, the process of matrix remodelling occurs to produce mature scar tissue. Part of this process involves reorganisation and contraction of existing collagen fibres. Contradictory reports exist in the literature regarding the influence of PHT on fibroblast-mediated collagen and wound contractions, depending on the *in vitro* [61, 68, 69] and *in vivo* [46] models used. PHT was shown to reduce fibroblast-mediated collagen gel contraction in human dermal fibroblasts [69], and this result was well correlated *in vivo* [70]. Phenytoin dose-dependently reduced wound contraction when applied as a powder to full-thickness porcine wounds. However, there was in no case any reduction in the rate of filling of the dermal discontinuity and, therefore, no impairment of healing. In effect, PHT appeared to increase matrix production to compensate for the reduced contraction, a process normally needed to reduce the total matrix synthesis required to close the wound.

FUTURE PROSPECTS

Currently, much attention and faith are placed on the development of expensive, topical molecular factors. The efficacy of such agents remains to be evaluated in clinical trials, and they may not prove to be useful. This could be due to the fact that these agents may not remain active when placed into a hostile wound environment; moreover, no single factor could be the answer to remedy a chronic wound [71]. PHT on the other hand is cheap and is readily available in most countries. Clinical studies using topical PHT therapy have suggested that it may be useful for the treatment of both acute and chronic wounds of various aetiologies in patients from diverse ethnic backgrounds. Its effectiveness, therefore, indicates that most individuals possess in their skin either enzymes capable of converting PHT to an active species or, alternatively, there are subpopulations of skin cells capable of responding directly to PHT itself. This is in contrast with the incidence of PIGO (approximately 50% of treated patients), which may be linked to differential individual metabolic capacities by

* Talas G and Brown RA, Manuscript submitted for publication.

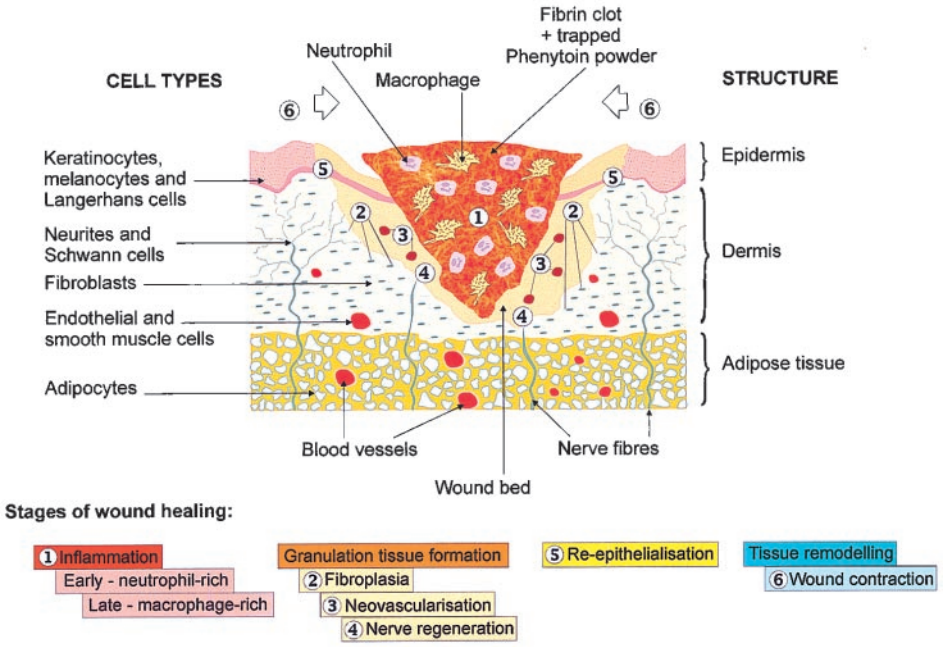

FIG. 6. Schematic overview of wound healing. *In vivo* and clinical studies suggest that topical PHT accelerates the inflammatory process, granulation tissue formation, and re-epithelialisation. Interestingly, it also reduces wound contraction while up-regulating matrix synthesis, and thereby healing is not impaired.

liver enzymes and the presence of responder cell populations in gingival tissues.

Although these results are encouraging, the efficacy of topical PHT therapy has yet to be confirmed by double-blind, placebo-controlled studies. Future studies would also seem to be usefully aimed at the production of various formulations (of PHT and derivatives) suitable for applications during the changing course of wound healing.

It is clear, that no single target/cell responsible for accelerated healing of PHT-treated wounds could be isolated (Fig. 6). Also, it is likely that PHT has direct and indirect effects on a variety of cells. More importantly, the active enantiomer of the pharmacophore needs to be identified for wound healing. This also raises the question, is it possible to design a new generation of safer, hydantoin-related drugs by replacing the phenyl rings without losing their activity? Future work to address this question will require the development of assay systems relevant to specific cell processes in wound healing. In the meantime, the study of both drug-induced gingival overgrowth and the mechanistic role of PHT in wound healing will give further insight into aspects of fibrosis, its prevention, and its enhancement in wound healing.

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