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Review

Iron as the malignant spirit in successful ageing

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Abstract

Iron enhances the production of the highly reactive and toxic hydroxyl radical, thus stimulating oxidative damage. Iron has been associated with a number of oxidative injury-dependent, age-related conditions and diseases. Indeed, oxidative injury is a major factor of (accelerated) ageing. This commentary reviews part of the existing literature on iron's deleterious effects, particularly in the context of ischemia-reperfusion injury and cardiovascular, brain and muscle diseases as well as skin ageing. Furthermore, the advantages of iron chelation are presented. Indeed, iron chelation or deprivation has been shown to act as a potent anti-oxidant in a variety of animal models of human diseases, preventing oxidative stress to tissues and organs. Iron chelators favor successful ageing in general, and when applied topically, successful skin ageing. It has also been proposed that gender-related differences in iron status are responsible for the increased longevity of women as compared to men. Despite this evidence, the role of iron in ageing and the possibilities of pharmacologically targeting iron have remained essentially unexplored. Iron thus appears as the "malignant spirit" in successful ageing.
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Keywords: Iron; Iron chelators; Reactive oxygen species; Hydroxyl radical; Fenton reaction; Successful ageing; Skin ageing

1. Introduction

Human societies today are faced with the challenges derived from ageing populations. Indeed, the recent evolution of age pyramids worldwide indicates a shift towards the elderly

Abbreviations: AD, Alzheimer's disease; DFO, desferrioxamine; DMD, Duchenne's muscular dystrophy; DPVR, diastolic pressure–volume ratio; FDO, 2-furildioxime; H_2O_2, hydrogen peroxide; OH, heme oxygenase; HSP, heat shock protein; LDL, low density lipoproteins; MMP, matrix metalloproteinase; $^{\bullet}OH$, hydroxyl radical; O_2, molecular oxygen; $O_2^{\bullet-}$, superoxide anion; ROS, reactive oxygen species; SOD, superoxide dismutase; UV, ultraviolet

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(>65 years old): in the past 50 years, life expectancy has increased by 50% (Bongaarts, 1998). Furthermore, a relatively new social group has emerged, called the "old elderly," those 85 years old and over, the fastest-growing group of Americans (Garry, 2001). American census data indicate that this group grew 274% between 1960 and 1994, whereas the "elderly" group grew by 100% during the same time period (Garry, 2001). In the near future even more than today, both of these age groups should continue to grow in quantitative and social importance. While the causes of this increase in lifespan remain misunderstood (improvements in health practices, hygiene, and living conditions can only partially explain the shift), interventions to modify it are limited, since the fundamental values of our society do not allow us to even contemplate a reduction in either longevity or the number of elderly people, and attempts to generate a compensatory increase in the number of infants and youth have to date been ineffective.

It is thus of utmost importance to understand and promote successful ageing, which is dependent on modifiable risks having positive effects on the ageing process (Garry, 2001). The most important among these modifiable risk factors are generation of and exposure to reactive oxygen species (ROS) and subsequent oxidative injury. On the one hand, ROS significantly contribute to both general and skin ageing processes, and on the other, their effects can be minimized effectively. The focus of this review will be iron, its role in the potentiation of ROS-mediated oxidative injury, the acceleration of various ageing processes and age-related diseases, and the potential of its removal, by iron chelation or deprivation, for both treatment and prevention of further ageing. Indeed, iron significantly contributes to the production of ROS via the non-enzymatic Haber-Weiss[1] and Fenton[2] reactions, producing the highly toxic $^{\bullet}OH$ radical (Halliwell and Gutteridge, 1984). Iron chelation and/or controlled deprivation may thus provide an effective therapeutic approach to various conditions associated with oxidative stress and ageing (Herschko, 1994).

Successful ageing involves physical, mental as well as social well-being (Kreps et al., 1999). As the quality of the appearance of the elderly and old elderly contributes to their well-being and continued social integration, successful skin ageing is an essential component of general successful ageing. Furthermore, the skin provides an interesting model or in vivo laboratory for the investigation of ageing processes. In this review, skin ageing will thus be discussed in some detail.

2. Oxidizing theory of ageing and oxidant/anti-oxidant equilibrium

Among the more than 300 theories on ageing, the one which today seems to be most prone to intervention is the one presenting ROS as a major culprit in ageing. This theory is based on the existence of an equilibrium and lack thereof between aggressive oxidants and defensive/protective anti-oxidants, and on the evidence that many age-related diseases or diseases known to accelerate ageing are caused by increased production or exposure to oxidants and subsequent oxidative injury (Kowald and Kirkwood, 1996; Mezzetti et al., 1996; McCord, 1998).

$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^-$ (iron catalyzed).

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$.

During normal metabolism, molecular oxygen (O_2), indispensable to the survival of aerobic species, is metabolized into reactive and potentially toxic ROS, via successive reductions, from O_2 to $O_2^{\bullet -}$, H_2O_2 and $^{\bullet}OH$. These reactions are catalyzed by a series of enzymes involved in the modulation of oxidative stress, such as superoxide dismutase (SOD), glutathione peroxidase and catalase, by the availability of cofactors of these enzymes such as selenium, and by iron. Iron, like oxygen, is indispensable for cell metabolism and in particular mitochondrial function. However, iron also plays a major role in oxidative stress via Fenton chemistry, where iron(II) is stoichiometrically oxidized by H_2O_2 to iron(III), producing the highly damaging oxygen radical $^{\bullet}OH$ (Gutteridge and Halliwell, 2000). Thus, among the ROS, the highly reactive $^{\bullet}OH$ is produced whenever (and wherever) iron is available for the Haber-Weiss and Fenton reactions, which is particularly relevant in the mitochondria, where normal cellular respiration, associated with the production of $O_2^{\bullet -}$ and H_2O_2, provides the substrates, in the presence of iron, for $^{\bullet}OH$ generation.

In small quantities, ROS in the human body are a natural by-product of cellular metabolism, to which they contribute. At such low concentrations, ROS may act as second messengers, gene regulators, and mediators for cell activation (Polla, 1999). Increased ROS production, however, becomes potentially damaging to almost every and all cellular and extra-cellular components, including proteins (intracellular as well as extracellular protein alterations), lipids (lipid peroxidation), DNA (strand breaks, cross-links, ...), and mitochondria (uncoupling of oxidative phosphorylation) (Takahashi and Niki, 1998) (Fig. 1). All of these lesions may be potentiated by iron (Fig. 1).

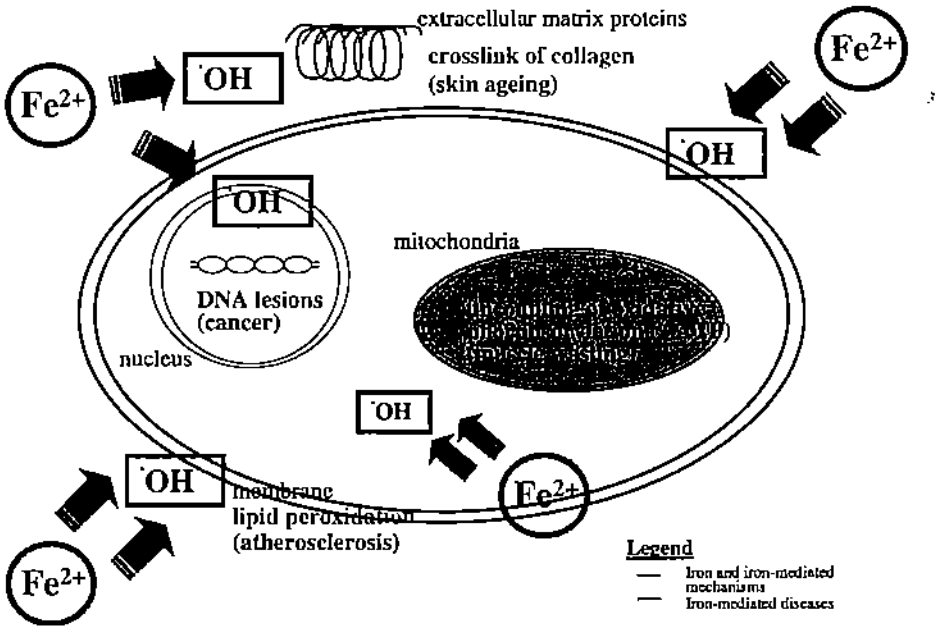

Fig. 1. Deleterious effects of iron on cellular components.

Increases in ROS may result from environmental exposures, including tobacco smoke, non-ionizing or ionizing radiation, or from pathological conditions such as ischemia-reperfusion, infection or inflammation. These conditions are linked to a variety of diseases, whether genetically determined (Pennisi, 1996; Odetti et al., 1998) or age-related, some of which are presented in Table 1 (adapted from Cross et al., 1987).

Iron has been shown to contribute to oxidative stress and the pathological process of many of these diseases (Toyokuni, 1996). Myocardial ischemia-reperfusion (Fig. 2) and UV exposure have been the subject of most studies on the potentiating deleterious effects of iron.

The participation of iron in cardiovascular diseases is exemplified by the fact that common mutations in the hemochromatosis genes such as the Cys282Tyr mutation, leading progressively to iron overload, present an increased risk for cardiovascular diseases, which has been reported by two independent European studies, one in women and one in men (Roest et al., 1999; Tuomainen et al., 1999; Garry, 2001). Thus, carriers of the Cys282Tyr mutation may represent an in vivo model for the study of the effects of iron overload in humans.

Table 2 lists various sources of major ROS and protective factors within cells. Indeed, eukaryotes are equipped with a genetically determined anti-oxidant store, which consists not only of SOD, catalase, and glutathione peroxidase, but also of more complex redox or protective systems such as thioredoxine, metallothioneins, and heat shock/stress proteins (HSP) (Yaagoubi et al., 1998; Yodoi et al., 1998).

The levels of the anti-oxidant enzymes and protective proteins can be regulated genetically, leading to inter-species variations as well as random variations between individuals, or through lifestyle (Favatier et al., 1997). Lifestyle may either participate in oxidant exposure (tobacco smoke, ultraviolet (UV) radiation), or contribute to the replenishment of anti-oxidant stores by an adequate nutrition or nutritional supplements (Ames, 1999).

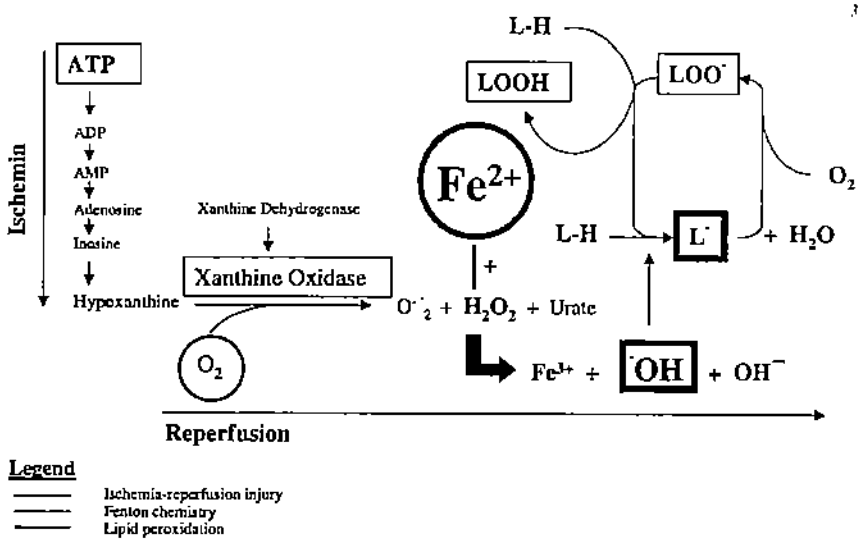

Fig. 2. Proposed central role of iron in ischemia-reperfusion-dependent lipid peroxidation.

Table 1
Examples of clinical conditions in which reactive oxygen species are involved

Multiorgan involvement
Ischemia-reperfusion states
Iron overload
Idiopathic hemochromatosis
Dietary iron overload
Thalassaemia
Ageing
Disorders of premature ageing (Werner's syndrome, Down's syndrome, ...)
Drug and toxin-induced reactions
Inflammatory-immune injury
Respiratory distress and multiple organ failure syndromes
Radiation injury
Cancer
Single organ involvement
Heart and cardio-vascular disease
Ischemia-reperfusion injury
Myocardial infarction
Brain
Ischemia-reperfusion injury
Alzheimer's disease
Parkinson's disease
Neurotoxins
Eye
Cataractogenesis
Retinopathy of prematurity
Lung
Cigarette-smoke effects (toxin-induced reaction)
Emphysema
Cancer
Muscle
Mitochondrial diseases
DMD
Kidney
Ischemia-reperfusion injury
Gastrointestinal tract
Endotoxin liver injury
Skin
Photoaging

Furthermore, iron levels also depend on both intake (nutrition, tobacco smoke) and on the levels of endogenous iron binding proteins such as ferritin or transferrin, which are in turn modulated by both ROS and iron (Rouault and Klausner, 1996; Stohs et al., 1997; Bomman et al., 1999).

Table 2
Major sources of ROS and protective factors within cells

Endogenous sources	Exogenous sources
Sources of ROS	
Mitochondrial electron transport chain	Sunlight
Microsomal electron transport chain	Ionizing radiation
Pro-oxidant enzymes	Redox-cycling substances
Lack of anti-oxidant enzymes or cofactors	Pro-oxidant drugs
Phagocytic cells	Cigarette smoke
Autoxidation reactions	
Iron	*Iron*
Protective factors	
SOD	Vitamin A
Catalase	Vitamin C
Glutathione	Vitamin E
Metallothioneins	Selenium
Thioredoxine	Zinc
HSP	
Iron-binding proteins (ferritin, ...)	*Iron chelators (natural, pharmacological)*

Exposure to oxidizing stress, endogenous or exogenous, stimulates the activity of endogenous anti-oxidants. During ageing, these endogenous anti-oxidant stores will be depleted, suggesting that exogenous anti-oxidants become more critical in older individuals. Key among exogenous anti-oxidants are those derived from the ingestion of nutrient-rich foods (Ames, 1999). Indeed, large epidemiological studies have recently shown that the ingestion of anti-oxidants of vegetable and fruit origin prevent certain diseases linked to ROS, increase lifespan, and contribute to successful ageing, which depends on a subtle equilibrium between oxidants and anti-oxidants. Iron plays a negative role in this equilibrium and overall tends to accelerate the onset and development of various age-related diseases. It has been further suggested that decreased iron stores contribute to increased life expectancy (Polla, 1999). Indeed, the increased longevity of women as compared with men might be the result of chronic iron deficiency, caused by menstruation and childbirth.

Successful ageing of the skin, a model for other oxidant-accelerated ageing processes, is thus based on a subtle equilibrium between oxidants and anti-oxidants (Mariéthoz et al., 1998). As such, if indeed oxidative stress and progressive oxidant/anti-oxidant imbalance play significant roles in ageing, then iron deprivation might prevent much of the cellular, tissue and organ lesions associated with ageing, thus contributing to successful ageing. Indeed, the involvement of iron in ageing has been established in many studies using iron chelation or iron deprivation as the experimental approach.

3. Protective effects of iron chelation or deprivation

The central role of iron in oxidative stress, and the ability of iron deprivation to prevent the generation of the highly toxic $^{\bullet}OH$, provide a strong argument for the use of iron deprivation and iron chelators as a therapeutic approach to various diseases mediated by

oxidative stress. We will concentrate on disorders in which iron chelation or deprivation has proven useful, including ischemia-reperfusion-dependent cardiovascular injuries, eye, brain and muscle diseases, and skin ageing.

3.1. Ischemia-reperfusion injury

Not only have high iron stores been associated with excessive risk of myocardial infarction, but data suggest that decreased levels of iron may prevent certain diseases and undesirable alterations (Salonen et al., 1992; Tuomainen et al., 1998). The benefits of iron chelation in the context of cardiac and vascular ischemia-reperfusion lesions have been extensively investigated. Qayumi and coworkers assessed the effects of iron depletion on ischemia-reperfusion injury of the spinal cord in a swine model of thoracic and thoracoabdominal aortic crossclamping (Qayumi et al., 1992). Animals were exposed to 30 min of ischemia that induced lethal and irreversible injury and paralysis. The control group received no pharmacological intervention, while the other group received 50 mg of desferroxamine (DFO). The methods of assessment were modified Tarlov criteria and blood flow analysis by radiolabeled microspheres. The animals treated with DFO had a significantly ($P < 0.05$) better neurological recovery than the control group. Indeed, when neurological assessment was performed four hours after reperfusion, 100% of the animals in the control group were paraplegic, whereas in the DFO-treated group, 14% had good movement without resistance (movement of the limbs without the ability to stand and walk) and 86% had good movement against resistance (they were able to stand and even walk with some difficulty). The authors concluded that the use of iron-chelating agents such as DFO appears promising in the protection of the spinal cord from ischemia-reperfusion injury.

Nakamura and coworkers studied the age-related differences in the effects of DFO in isolated rabbit hearts subjected to ischemia and reperfusion (Nakamura et al., 1992); DFO was administered just prior to reperfusion. Results were assessed by measuring isovolumic systolic and diastolic function, and the content of adenine nucleotides (ATP, ADP, AMP). After 30 min of ischemia and 30 min of reperfusion, results showed that the diastolic pressure–volume relationship (DPVR) remained constant (at the pre-ischemic values) in both the untreated and the DFO-treated groups in newborn hearts. In adult hearts, the slope remained constant in the treated group, but rose significantly in the control group. After 40 min of ischemia, the DPVR slope rose in both newborn and adult hearts, the latter being greater in the control group versus the treated group. These results suggest that DFO significantly improves cardiac function only in adult hearts ($P < 0.01$), but significantly improves systolic function in both newborn and adult hearts ($P < 0.01$). The authors concluded that newborn hearts appear to recover post-ischemic function and metabolism faster than adult hearts and that DFO improves post-ischemic heart function in both age groups.

Iron chelators also play a key role in the development of atheromatous lesions. Indeed, iron and iron-driven $^{\bullet}OH$ contribute to the oxidation of low density lipoproteins (LDL) and lipo-peroxidation in general, which when occurring within vessel walls, is an important step in atherogenesis. The role of iron in the initiation/acceleration of the oxidative modification of LDL has been studied by Yuan and coworkers. They designed an in vitro model where human monocyte-derived macrophages were exposed to UV-exposed erythrocytes (Yuan et al., 1996). The macrophages were then exposed to LDL, with or without DFO. The

capacity of macrophages to oxidize LDL was much enhanced following erythrophagocytosis. This process involved the release of iron, and indeed LDL oxidation was inhibited by DFO.

Despite this convincing literature, the effects of iron chelation have not been confirmed in all models. For example, in Nakamura's model of ischemia-reperfusion discussed previously (Nakamura et al., 1992), DFO failed to improve the recovery of myocardial ATP content, and in Jensen's study of cardiac function in non-thalassaemic patients with transfusional iron overload, although the decrease in cardiac function clearly related to the iron overload, the efficacy of iron chelation was not significant (Jensen et al., 1997).

Iron chelation exerts a beneficial cardiovascular effect in most situations of iron overload, whether genetically determined (such as in hemochromatosis) or caused by excessive transfusions. In both of these situations, iron chelation improves cardiac function, even in normal males. Indeed, a study has shown that healthy elderly individuals can donate up to three or four units of blood per year without negative effects (Garry et al., 1991). Because the link between excessive body iron stores and an increased risk of acute myocardial infarction has been established, yearly donations of one unit of blood to a blood center by those individuals with diagnosed abnormally elevated iron stores may be beneficial both to the individual and to the society, as the insufficient quantity of available blood is often a cause of sub-optimal treatment (Salonen et al., 1992; Tuomainen et al., 1998).

3.2. *Eye, brain and muscle diseases*

Despite the fact that corneal epithelial cells are constantly exposed to UV light and thus to ROS, primary cancers of these cells are extremely rare, which is in sharp contrast with corneal fibroblasts or skin cells (Smolinand and Thoft, 1987; Cai et al., 1998). A likely explanation for this observation is that corneal cells have developed specific protective mechanisms, a tight regulation of iron available for the Fenton reaction being one of them. Interestingly, corneal epithelial cells are much more resistant to UV-induced DNA strand breaks, as determined by an in situ 3′-end labeling method, than other corneal or non-corneal cells (Cai et al., 1998). When nuclear ferritin expression was inhibited in the corneal epithelial cells, or when ferrous sulfate was added to the culture medium, corneal cells lost their resistance to UV-mediated DNA strand breaks.

Iron has also been associated with the pathological lesions of Alzheimer's disease (AD) (Smith et al., 1997). Indeed, accumulation of iron was found to be associated with senile plaques and neurofibrillary tangles, indicating alterations of iron homeostasis in AD, which is further supported by the elevated serum levels of the iron binding protein p97 found in patients with AD (Kennard et al., 1996). Moreover, iron greatly increases the vulnerability of neurons to amyloid toxicity, which involves membrane lipid peroxidation resulting in the production of the aldehyde 4-hydroxynonenal, which, in turn, impairs the function of membrane ATPases and glucose and glutamate transporters (Goodman and Mattson, 1994; Mark et al., 1997a,b; Keller et al., 1997). As in other age-related diseases, iron deprivation might prove a useful adjunctive approach to suppress the evolution of brain lesions in AD.

Such an approach is further supported by the effects of iron deprivation in Duchenne's muscular dystrophy (DMD). DMD is a severe genetic disorder due to mutations in the dystrophin gene at Xp21, which prevents the expression of this cytoskeletal, membrane-

associated protein in affected patients, leading to progressive muscle wasting (Engel, 1986). Although DMD is not an age-related disease, the muscle wasting subsequent to the lack of dystrophin is biochemically oxidant-dependent, enabling us to consider this genetic disease affecting young boys as a model for age-related muscle wasting. Indeed, the reduction of iron stores via various methods have been investigated to treat DMD. Clark proposed that DFO be used, given its ability to prevent oxidant damage by removing iron (Clark, 1984). Clark suggested that use of this iron chelator in the context of DMD would indeed be consistent with the fact that DFO has been used in long-term dialysis patients. Clark argued that because of the ample evidence demonstrating that DFO prevents acute free-radical-induced oxidative processes that cause biological changes similar to those implicated in DMD, and because use of DFO presented no side effects, its use in DMD would provide a therapeutic advance. Another approach that has been tested in the context of this disease is dietary iron deprivation as a therapeutic method for reducing iron levels (Bornman et al., 1999). Results of this study indicated that dietary iron deprivation led to a significant decrease in the necrotic fibers in iron-deprived *mdx* mice. These results suggest that iron-driven generation of $^{\bullet}OH$ does play a role in the necrosis of dystrophin-deficient muscle fibers and possibly in all types of ROS-related muscle wasting.

4. Iron and skin ageing

The skin contains significant iron levels that may be available to participate in ROS formation, photodamage, and photoinduced skin ageing (Bissett et al., 1994; Bissett and McBride, 1996). It has been shown that UV exposure stimulates the production of ROS as well as the release of free iron, which in turn induces further production of ROS—a "malignant" vicious cycle (Aubailly et al., 1991; Bissett et al., 1991; Jurkiewicz and Buettner, 1994). Photodamage can be considered as an experimental in vivo model for skin ageing, and iron chelators able to prevent acute photodamage should also prevent photoinduced ageing.

Topical iron chelators such as 2-furildioxime (FDO) have been shown indeed to be efficient in the prevention of short-term photodamage. Topical 5% FDO application associated with 13% skin penetration led to a complete suppression of UV-induced erythema and a marked inhibition of sunburn cell formation in human skin (values of 6.8 and 0.15 for sites treated with vehicle and 5% FDO, respectively) (Bissett et al., 1994). FDO also delayed for 10 weeks the appearance of skin wrinkling in hairless mice exposed to UVB radiation and for 8 weeks the onset of skin tumors (papillomas and squamous cell carcinomas) (Bissett and McBride, 1996).

Topical iron chelators thus prevent both short-term and long-term UV-induced photodamage, further supporting their use in the prevention of skin ageing. Human dermal fibroblasts were UVB radiated and activation of Jun N-terminal kinase 2 (JNK2) as well as collagenase (matrix-degrading metalloprotease (MMP-1)) and stromelysin (MMP-3) mRNA levels were determined. Preincubation with FDO prevented both JNK2 activation and MMP-1 and MMP-3 mRNA increases, indicating again that iron-driven reactions are central to cellular UVB responses (Brenneisen et al., 1998).

Based on the observations by Bissett et al. (1991, 1994) and Bissett and McBride (1996) that topical application of iron chelators delayed the onset of visible and histological skin

changes induced by long-term sub-erythemal doses of UVB radiation, Mitani et al. (2001) have proposed that bifunctional substances (both anti-oxidants and iron chelators) should be effective for photoprotection. Kojic acid has both scavenging and iron-chelating activity, which is also the case for many polyphenol flavonoids and in particular proanthocyanidines. Kojic acid significantly suppressed UVB-mediated lipid peroxidation and subsequently prevented wrinkling, hyperplasia of the epidermis, and fibrosis of the lower dermis in hairless mice (Mitani et al., 2001). The long-term topical use of non-toxic naturally occurring substances such as kojic acid and anthocyanines should be further tested in humans for the prevention of skin ageing (Noda et al., 1998).

5. Discussion and perspective

Schneider presented two possible scenarios emerging from the current ageing of the population—a catastrophic scenario (scenario 1) and an optimistic scenario (scenario 2) (Schneider, 1999). According to scenario 1, the levels of support for and investment in research, prevention, and treatment in the field of ageing are not appropriately increased, resulting in few, if any, improvements in the average health of the elderly. Consequently, the average health of an elderly person in the year 2040 will not be very different from that of a current elderly person, with substantial needs for acute and long-term care. According to scenario 2, levels of support for and investment in research, prevention and treatment in the field of ageing are increased appropriately, resulting in dramatic advances in the treatment and prevention of the major causes of disability in the older population. Consequently, the average health of an 85-year-old in the year 2040 will more resemble that of a current 70-year-old, with much reduced needs for acute and long-term care. Garry concurs and suggests that the number of successfully ageing older individuals will continue to increase only in the context of persistent recognition and modifications of those risk factors associated with age-related chronic disease (Garry, 2001).

Overall, the data reviewed here suggest that a tightly controlled iron decrease could promote successful ageing. Indeed, some studies even suggest that heterozygosity for Cys282Tyr in the human hemochromatosis gene inversely correlates with longevity (Beutler and Felitti, 2002; Lio et al., 2002). Given the body of available data, it is surprising that the pharmaceutical industry interested in successful ageing has not yet developed iron-based research programs. Iron chelation or deprivation appears to be feasible through the use of systemic chelation therapy or blood removal, two approaches that are unlikely to promote the rapid growth of an emerging market. Subcutaneous and oral preparations of iron chelators have not encountered long-lasting enthusiasm (Propper et al., 1977; Olivieri et al., 1990; Agarwal et al., 1992). It could be that the concept of "taking away" rather than supplementing is rather foreign to pharmacological R&D (Polla, 1999). We would suggest however that iron be removed from all vitamin or oligo-element-containing preparations recommended to elderly individuals. Furthermore, promotion of successful ageing by iron control could also benefit from lessons from the "skin laboratory." Indeed, iron chelation by kojic acid or other natural or synthetic iron chelators such as anthocyanines or FDO might well pave the way for a broader application of such a simple preventive approach which should tame the "malignant spirit" (Noda et al., 1998).

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