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Genistein: a natural isoflavone with a potential for treatment of genetic diseases

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Abstract

Genistein [4′,5,7-trihydroxyisoflavone or 5,7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one] is a natural isoflavone occurring in many plants known to possess various biological activities, ranging from phyto-oestrogenic to antioxidative actions. Recent studies indicated that this isoflavone can also be considered as a drug for as yet untreatable genetic diseases. In the present review, we discuss a plausible use of genistein in treatment of two genetic disorders: CF (cystic fibrosis) and MPS (mucopolysaccharidosis). Although various biological actions of genistein are employed in these two cases, *in vitro* studies, tests on animal models and pilot clinical trials suggest that this plant-derived compound might be a real hope for patients suffering from severe inherited disorders with relatively complicated pathomechanisms, including those affecting the central nervous system.

Introduction: genistein as a multifunctional bioactive compound

Genistein (Figure 1) is a common name of 5,7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one. Since this compound belongs to the group of isoflavones, heterocyclic polyphenols that naturally occur in plants, it is also called 4′,5,7-trihydroxyisoflavone [1]. In fact, some plants, including soya, are especially rich in genistein, although in the natural form it occurs as a glycoside called genistin (Figure 1), rather than as an aglycone (for a review, see [2]).

There have been many biological functions of genistein reported to date. Among them, phyto-oestrogenic, antioxidant and tyrosine kinase inhibitor activities are the most intensively studied (for reviews, see [1,3]). Since these activities, although broad, were described as relatively weak, for a long time genistein has generally been considered as an auxiliary medicine, used in supportive treatment, rather than as a main therapeutic. However, its low toxicity [4] and undoubtedly significant (although not dramatic) biological effects [3] have encouraged studies in which it has been tested as a potential protective and/or therapeutic agent in a variety of disorders, including cancer [5–7], cardiovascular diseases [8,9] and menopausal symptoms [10,11]. Moreover, it has been discovered that genistein also has other biological activities, therefore researchers have started to investigate whether this isoflavone might be used in the treatment of other diseases. One example is its antimicrobial potential [12,13], and thus a possible use of genistein is in the treatment of bacterial infections, which is especially important in the current antibiotic crisis caused by the rapid appearance of multidrug-resistant pathogenic bacteria. Since this aspect has been reviewed previously [14], we will not focus on it in the present review. On the other hand, another therapeutic aspect of genistein is considered, namely recent studies have suggested that genistein may be a drug for treatment of some genetic diseases. Therefore this apparently unexpected medical role of genistein is highlighted in the present review.

Genistein as a potential drug in CF (cystic fibrosis)

CF is the most common fatal genetic disease in populations of the U.S.A. and Europe. In these populations, the frequency of this disorder is higher than 1 per 3000 live births, with a carrier frequency of approx. 1 in 25 among Caucasians (for recent reviews and commentaries, see [15–17]). Despite recent achievements in management of symptoms and improvement of life expectancy of patients, CF is still a fatal disease, with an average lifespan in the U.S.A. and Europe still below 40 years [16]. The main clinical problem occurring in CF is chronic inflammation of airways, caused by frequent and severe

Key words: cystic fibrosis, gene expression-targeted isoflavone therapy, genistein, isoflavone, mucopolysaccharidosis, substrate reduction therapy.

Abbreviations used: BBB, blood–brain barrier; CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator protein; CNS, central nervous system; EGF, epidermal growth factor; EGFR, EGF receptor; ERQC, endoplasmic reticulum quality control; ERT, enzyme-replacement therapy; GAG, glycosaminoglycan; GET IT, gene expression-targeted isoflavone therapy; HSCT, haemopoietic stem cell transplantation; LSD, lysosomal storage disease; MPS, mucopolysaccharidosis; SRT, substrate-reduction therapy.

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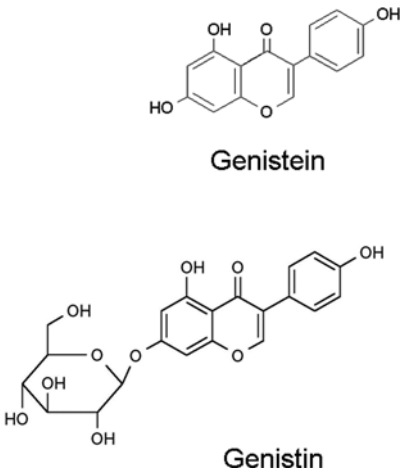
Figure 1 | **Genistein and its glycoside, genistin**



bacterial infections. This leads to the progressive suppurative pulmonary disease, which results in airway obstruction, destruction and respiratory failure [16]. At later stages of the disease, airway inflammation can be associated with a systemic inflammatory response. Although the lung disease is not the only symptom of CF patients (for example, pancreatic dysfunction, elevated sweat electrolytes and male infertility are also common in these patients), the chronic pulmonary dysfunction is the main cause of morbidity and mortality [15,16].

The clinical problems in CF arise from dysfunction of a single gene. CF is a monogenic disease, inherited in an autosomal recessive manner [15–17]. The gene affected in patients suffering from this disease is *CFTR*, which encodes the CF transmembrane conductance regulator protein. The CFTR protein functions as an epithelial Cl^- channel, whose activity is regulated by cAMP-dependent protein kinase A and ATP. In a lack or decreased activity of CFTR, the transport of Cl^- ions is impaired, which results in dehydration of endobronchial secretions and cripples mucociliary clearance [15,16]. Under such conditions, airways may be obstructed, and bacterial cells, which normally should be eliminated from the lung, remain in the pulmonary system causing infection. Moreover, abnormal airway surface liquid volume, resulting from impaired electrolyte transport, causes further problems with bacterial clearance, increasing probability of severe infections [16].

Although several hundred mutations in the *CFTR* gene have been described to date [15], between 70 and 90% of CF patients (depending on population) bear the most frequent defective allele of this gene, called ΔF508. This mutation leads to a deletion of a phenylalanine residue at position 508 in the 1480-amino-acid polypeptide, which has two dramatic effects on the protein properties. First, the product of the mutant allele has severe problems with proper folding. This causes its recognition by the ERQC (endoplasmic reticulum quality control) system, and exposition to proteolytic degradation. As a consequence, little, if any, ΔF508 CFTR protein reaches the cellular membrane, the normal compartment of wild-type CFTR function. Secondly, the activity of ΔF508 CFTR as a Cl^- ion channel is severely impaired. Therefore the mutated protein is not able to both reach its proper localization in the cells and perform its biochemical function [15].

The frequency of the ΔF508 allele and mechanisms of dysfunction of its product encouraged researchers to look for potential pharmaceuticals that might restore, at least partially, the proper localization and biochemical function of the mutant protein. Among other strategies (reviewed recently in [18]), the use of two novel classes of drugs appears to be especially promising.

The first class encompasses compounds called 'correctors' that may allow the mutant CFTR protein to bypass ERQC and reach the cellular membrane. Thus any residual activity of the properly located protein may cause more or less efficient transport of Cl^- ions, which should significantly improve functioning of affected tissues and organs. Different chemicals, including 4-phenylbutyrate, curcumin and an analogue of sildenafil, are currently tested for their functions as correctors of the ΔF508 CFTR protein [18].

'Potentiators' are the second class of putative drugs for CF patients. These compounds can enhance residual activity of the properly located mutant protein, perhaps by acting as small molecular chaperones [18]. Genistein is one of such chemicals. This isoflavone can enhance the Cl^- ion channel activity of not only ΔF508 CFTR [19], but also the product of another *CFTR* allele, G551D (present in 2–6% of CF patients [18]), which may be especially amenable to this kind of treatment as it reaches the plasma membrane as a mature protein, but has severely impaired biochemical function [20]. It appears that the action of genistein on CFTR is direct [21,22].

Although several other potentiators of ΔF508 CFTR were found [23,24], genistein is still one of the most efficient activators of the mutant protein, and, because of its low toxicity [4], it can be placed among the best candidates for a CF therapeutic. In fact, genistein has been demonstrated to be more effective in partial restoration of the ΔF508 CFTR activity than other potentiators, including scopoletin, isopsoralen, osthole, imperatorin, praeruptorin A and UC_{CF}-029 (7,8-benzoflavone) [23,24].

Interestingly, recent studies on the mechanism of genistein-mediated potentiation of ΔF508 CFTR revealed that this isoflavone acts not only by Cl^- channel gating. Moderate concentrations of genistein augmented CFTR maturation and increased its localization to the cell surface [25]. These results indicate that genistein may be an even more potent drug in the treatment of CF than suggested previously, as it may enhance expression, proper localization and activity of the mutant protein.

The encouraging results of pre-clinical studies with genistein provide a basis for clinical trials with CF patients. In fact, the Phase II clinical trial with a combined treatment (4-phenylbutyrate and genistein) of CF patients with the ΔF508 mutation has commenced [18].

Genistein as an experimental drug in Sanfilippo disease (mucopolysaccharidosis type III) and a potential therapeutic agent for other LSDs (lysosomal storage diseases)

LSDs are inherited metabolic disorders caused by dysfunction of enzymes involved in either degradation of particular compounds in lysosomes or lysosomal transport [26]. There are 50 or so known LSDs, which are grouped into severe and progressive disorders.

MPSs (mucopolysaccharidoses) are classical examples of LSD. Storage of GAGs (glycosaminoglycans) in cells of patients suffering from MPSs results in progressive damage of the affected tissues, including the heart, respiratory system, bones, joints and, in some cases, CNS (central nervous system) [27]. Depending on the lacking or deficient enzyme and kinds of accumulated GAGs, 11 types and subtypes of MPS have been recognized [27]. Apart from MPS II, which is an X chromosome-linked disease, all other MPS types are inherited in an autosomal recessive manner. In most cases, these diseases are fatal, with an average expected lifespan of one or two decades.

Until 2003, no effective treatment was available for any MPS type. Currently, ERT (enzyme-replacement therapy), based on an intravenous infusion of an active recombinant form of a deficient enzyme, can be used for treatment of MPS I, MPS II and MPS VI [28]. This therapy is effective in treatment of somatic symptoms. However, neurological symptoms developed due to GAG accumulation in the CNS cannot be managed by ERT owing to an inefficient delivery of proteins through the BBB (blood–brain barrier). The CNS dysfunction-related symptoms occur in some MPS I patients (subtype MPS IH), most MPS II and MPS VII patients, and all MPS III patients [27], where they are especially severe.

Sanfilippo disease (MPS III) is a group of four conditions (MPS III subtypes A, B, C and D), revealing similar clinical symptoms and characterized by lysosomal storage of HS (heparan sulfate), a GAG [27]. This condition is associated with severe learning difficulties and behavioural disturbances, and with relatively mild somatic involvement. In most affected patients, the progressive nature of the disease leads to death in the second (or rarely third) decade of life [27]. As this disorder primarily affects the brain and nervous system, attempts to cure the patients have not been successful, and the best that can be offered is palliative or symptomatic care.

An analysis of the regulation of metabolism of GAGs has led to the suggestion that SRT (substrate-reduction therapy), which is based on an impairment of production of substrate(s) that cannot be degraded due to defects of particular enzyme(s), might be an alternative solution for patients suffering from MPSs, particularly from their neuronopathic forms [29]. However, since GAGs are synthesized from monosaccharides (e.g. galactose, xylose, *N*-acetylglucosamine and others) that are also involved in many other biochemical pathways, a potential use of their analogues as competitive inhibitors of certain synthetases might cause defects in various biochemical reactions in cells and resultant side effects in patients. It was therefore proposed that, in the specific case of MPS, regulation of expression of genes coding for particular enzymes required for GAG synthesis might be a primary target for SRT rather than activities of these enzymes [29].

Early observations have shown that maximum synthesis of some GAGs requires either follicle-stimulating hormone or EGF (epidermal growth factor) [30]. EGF influences expression of certain genes by binding to its transmembrane receptor that upon this interaction becomes an active protein kinase, initiating a specific kinase cascade that finally results in regulation of activity of particular transcription factors (Figure 2). This tyrosine-specific protein kinase activity of the EGFR (EGF receptor) is inhibited by genistein [31]. Thus one might assume that, in the presence of genistein, GAG synthesis might be impaired. Moreover, since this isoflavone can cross the BBB to some extent [32], it was reasonable to test whether addition of genistein may cause a reduction in GAG production.

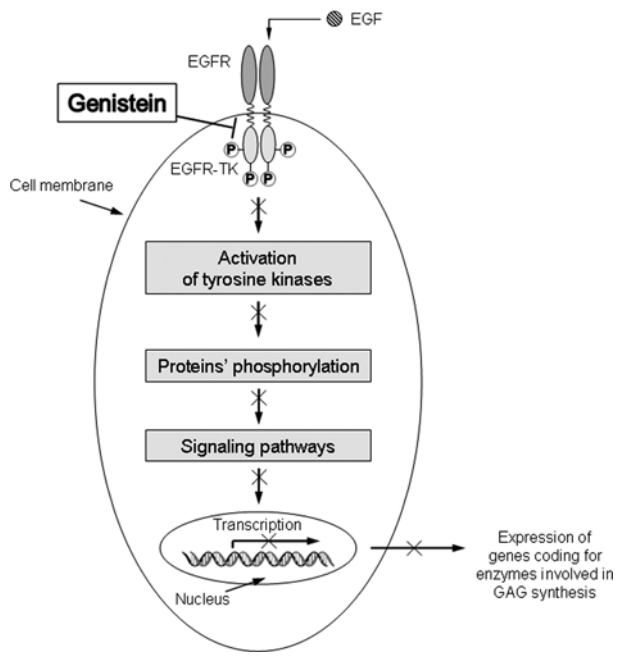
When genistein was present in the medium of cultured human fibroblasts, it inhibited GAG synthesis in cells of MPS I, MPS II, MPS IIIA and MPS IIIB patients (as measured by incorporation of a radioactive precursor) [33]. This inhibition has led to a decrease in lysosomal GAG storage, observed after 1 week of incubation of fibroblasts with genistein at concentrations between 10 and 30 μM (this was determined biochemically, by measurement of actual intracellular GAG levels, and in electron microscopic studies, by observations and counting of untypical intracellular structures) [33].

Studies on the mechanism of genistein-mediated inhibition of GAG synthesis confirmed the theoretical prediction [33] that the main regulatory pathway affected in this biological system is the signal transduction pathway initiated by the protein phosphorylation reaction stimulated by EGF interaction with its receptor (Figure 2). Namely, an excess of EGF abolished genistein-mediated impairment of GAG synthesis in cultured fibroblasts, whereas increased concentrations of genistein partially restored this negative regulation [34]. Moreover, genistein affected EGFR-catalysed phosphorylation efficiency [34]. These results, together with the finding that efficiency of GAG synthesis may significantly influence the severity of MPS diseases [35], support the validity of the name proposed for the therapy based on the use of genistein in MPS: GET IT ('gene expression-targeted isoflavone therapy').

A recent pilot clinical study with ten patients suffering from MPS IIIA and MPS IIIB indicated that genistin-rich soya isoflavone extract, administered orally for 12 months at the dose corresponding to 5 mg of genistein per 1 kg of body weight daily, caused a statistically significant improvement in all parameters tested [36]. Particularly, after 1 year of therapy, urinary GAG levels were reduced, hair morphology (assessed to be a useful parameter in monitoring efficacy of

Figure 2 | A mechanism for genistein-mediated impairment of expression of genes, whose products are involved in GAG synthesis

Stimulation of expression of genes coding for enzymes required for GAG production depends on EGF-mediated activation of its receptor, EGFR. The EGFR tyrosine kinase (EGFR-TK) activity causes phosphorylation of certain proteins involved in the cascade of kinases, which is the process of transduction of intracellular signals, leading to activation of transcription of specific genes in the nucleus. Inhibition of the EGFR-TK activity by genistein (thick blunt-ended line) results in impairment of the signal transduction and thus in a decreased expression of genes coding for enzymes involved in GAG synthesis. Therefore the secondary effect of this negative regulation is decreased efficiency of GAG production.



treatment of patients suffering from various MPS types [37–39]) improved, and patients achieved higher scores in the psychological test that was constructed to measure cognitive functions [36]. The last result is especially important as this was the first report demonstrating an improvement of MPS III patients after pharmacological treatment, and one of the first, if not the first, direct indications that neurological functions, already lost due to neurodegeneration in the course of a disease, can be restored (at least partially) after pharmacological treatment [40]. Moreover, an improvement or stabilization in several non-quantified clinical parameters, based on subjective assessments, was reported (Table 1) [36]. It is worth noting that, owing to a progressive character of this disease, a gradual deterioration of most clinical parameters is observed in untreated MPS III patients [27]. Importantly, no significant side effects were observed during the GET IT clinical study [36], which indicated that this treatment is safe.

The encouraging findings described above have been corroborated by recent results of studies on both cellular and animal models of Sanfilippo disease, where either genistein or another inhibitor of GAG synthesis, rhodamine B, were used [41–43]. Furthermore, RNA interference-mediated specific silencing of chosen genes coding for enzymes involved in GAG synthesis resulted in inhibition of GAG production [44,45], indicating that gene expression-targeted therapies may be effective in treatment of MPS indeed. Thus further clinical studies on the use of genistein in the treatment of MPS III patients appear to be highly desired, and, in fact, a double-blinded placebo-controlled clinical trial, with the use of the soya isoflavone extract at the dose corresponding to 10 mg of genistein per 1 kg of body weight daily, has been initiated in June 2009 (F. Wijburg, personal communication).

Since accumulation of GAGs also occurs in some other LSDs (e.g. mucolipidoses, multiple sulfatase deficiency) [26,27], it appears that further studies on the mechanisms of LSDs and activities of genistein and other flavonoids should be important in development of novel therapeutic procedures for as yet non-treatable genetic diseases. One might also consider that a combination of two (or more) different therapies, for example ERT and GET IT, can be more effective than any single therapy. It is particularly tempting to speculate that such a combination may be especially suitable for neuronopathic forms of MPS (e.g. MPS IH, severe forms of MPS II), for which ERT is effective only in the treatment of visceral organs. In fact, a combination of ERT and HSCT (haemopoietic stem cell transplantation) was tested recently as a therapy for MPS IH (Hurler's syndrome) [46], and we believe that it is reasonable to propose a combination of GET IT with ERT or HSCT or both, which might improve the efficacy of treatment of MPS.

Since in the pilot clinical study with GET IT for MPS III a soya isoflavone extract was used rather than pure genistein [36], a question appeared as to whether a natural source of this isoflavone or a synthetic compound should be the final form of the drug. In most soya extracts, genistein occurs in the form of its glycoside, genistin. Since bioavailability of genistein was reported to be higher than genistin in rats [47], further clinical studies with pure genistein appear to be required.

If we consider the use of isoflavone extracts, another question regarding GET IT is the source of the biologically active compound. Since various isoflavone-containing products are commercially available, it was crucial to determine their actual composition and quality. A previous study has indicated that, among products available in the U.S.A., actual content of isoflavones was between 41 and 99% of that claimed by manufacturers [48]. Over 50-fold differences in total amounts of isoflavones were found among various soya isoflavone supplements and extracts tested in Western Europe [49]. A recent report describes extremely different amounts of isoflavones determined in various products purchased in Poland, from 0.13 to 39 mg per tablet, although amounts declared by manufacturers were similar [50]. Moreover,

Table 1 | **Effects of 1-year treatment of MPS III patients with genistein-rich soya isoflavone extract (at the dose of 5 mg/kg of body weight per day) during the pilot clinical studies**

Data taken from [36].

	Patients revealing (after 1 year of treatment, relative to baseline)		
Parameter	**Deterioration (%)**	**Stabilization (%)**	**Improvement (%)**
Measured parameters*			
Urinary GAG level	30	0	70
Hair morphology	0	20	80
Cognitive functions	0	20	80
Estimated parameters†			
Speech performance	10	40	50
Speech comprehension	0	40	60
Activity	0	60	40
General behaviour	0	40	60
Sleep habits	0	30	70
Pain	0	90	10
Walking	20	40	40
Manual dexterity	0	60	40
Joint mobility	20	40	40
Breathing during day	0	80	20
Breathing at night	0	70	30
Infections	0	20	80
Hearing	0	70	30
Skin elasticity	10	30	60
Hair structure	10	10	80
Dyspeptic symptoms	0	50	50
Stools	10	10	80

*Values were measured using objective methods (biochemical assays, microscopic observations and psychological tests).

†Assessments were performed on the basis of subjective criteria (questionnaires and clinical observations).

only some of these products were found to be effective in inhibition of synthesis of GAGs in cultured fibroblasts [50]. Therefore a careful choice of a proper isoflavone extract is crucial for efficacy of GET IT.

Concluding remarks

Genistein, a natural isoflavone occurring in relatively large amounts in some plants (e.g. soya), appeared to be an effective therapeutic in inherited diseases, as different as the most frequent fatal genetic disease in the Caucasian population of U.S.A. and Europe, CF, and one of the most severe genetic diseases, MPS. Although molecular mechanisms of action of genistein in these two cases are different, multiple biological activities of this isoflavone make it an attractive compound when a safe and effective treatment of a complicated disease is considered. It appears that further studies on: (i) optimization of genistein-based therapies (particularly, optimization of the dose and the method of application); (ii) artificial derivatives of genistein, which might have even more desirable therapeutic properties; and (iii) combination of the use of genistein (or its derivatives) and other therapeutic methods may lead to development of highly effective medical procedures, allowing the management of as yet untreatable genetic diseases.

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