

Structurally Modified ‘Dietary Flavonoids’: Are these Viable Drug Candidates for Chemoprevention?

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Abstract: The field of chemoprevention continues to be a heavily researched area since a few decades now. Whereas, the dietary intake of flavonoids occur *via* daily intakes of fruits, vegetables, herbal preparations, beverages etc, it appears that the active ingredients(s) contained in these dietary sources may not reach the required plasma and/or tissue concentrations *in vivo* to produce the desired pharmacological response expected of these agents. Akin to the common problems of drug-gability encountered often times in drug discovery/development scenario, low bioavailability of flavonoids have been attributed to: lack of stability, excessive metabolism, permeability problems, lack of site specificity in distribution, rapid elimination etc. The scope of the review is to assess and put into perspectives salient features of some of the recently reported work on dietary flavonoids including the methylated compounds that showed improved drug-like properties in context with the required features for the lead optimization program rendering a clinical candidate. In addition, it aims to provide some perspectives into the present day considerations for early drug development such as healthy subjects versus cancer patients, single agent versus combination potential with other cancer therapeutics, selection of a cancer indication, potential for drug-drug interaction etc. Although there has been an unabated use of ‘dietary flavonoids’ with tall order claims for chemoprevention, it may be extremely challenging to confirm it in a clinical setting. Overall, it appears prudent to develop a comprehensive prospective strategy for clinical development and regulatory approval.

INTRODUCTION

The field of oncology has witnessed revolutionary changes with the introduction of novel targeted therapies and there appears to be a paradigm shift in the thinking process to keep a balance between the traditional cytotoxics and the new generation targeted therapies. A well articulated commentary provides a strong basis to view oncology development from a different perspective regardless of the nature of the compound being developed [1]. Amidst these new thoughts and ideologies surrounding the next phase of newer introductions of oncology compounds, chemoprevention strategies are making its rounds with lot more vigour and conviction than ever before – it is well known that active research in the field of chemoprevention has been rigorously pursued since a few decades now. Over the years, the fascination of using naturally occurring flavonoids has not lost its sheen [2-6].

Briefly, flavonoids are polyphenolic moieties and are extremely water soluble. Interestingly they display two or more cyclic ring (i.e., benzene type) structures. The flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins and isoflavonoids. Flavonoids are purported to be beneficial due to several medicinal value proposition they offer such as anti-cancer, anti-oxidant, anti-allergic, anti-viral etc. Some of the well known flavonoids include: rutin and quercetin found in red wine; hisperidin found in citrus fruits and natural beverages like black tea contain a very proportion of dietary flavonoids [7, 8].

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SCOPE

The scope of this article is to assess and put into perspectives salient features of some of the recently reported work on dietary flavonoids as it relates to multiple activities such as issues of druggability, influence of structural changes on the attributes, important development considerations – tissue distribution, metabolic assessment, interference with transporter systems, assessment of human bioavailability, specific issues related to methylated compounds. Also, provide some perspectives, in context with the required features for lead optimization and the present day considerations for early drug development.

FLAVONOIDS – ISSUE OF DRUGGABILITY

Whereas, the dietary intake of flavonoids occur *via* daily intakes of fruits, vegetables, herbal preparations, beverages etc, it appears that the active ingredients(s) may not reach threshold levels *in vivo* to elicit pharmacological response expected of these agents. The probable reasons associated with the lack of bioavailability of these dietary flavonoids is akin to the common problems of druggability encountered often times in drug discovery/development scenario for many programs. The reported issues for the lack of bioavailability for flavonoids were ranging from lack of stability, excessive metabolism, permeability problems, lack of site specificity in distribution, rapid elimination etc [9-13].

STRUCTURAL MODIFICATIONS OF FLAVONOIDS – KEY ATTRIBUTES

The introduction of methyl group(s) into the structure of flavones (i.e., leading to the formation of methoxylated flavones) provides some outstanding features hitherto non-existent with the free hydroxy flavonoids [14-17]. These

unique drug-like properties that provide differentiating features were as follows: a) resistance to hepatic conjugative metabolism: the ease of Phase II conjugation *via* glucuronic acid and/or sulfation pathway(s) is prevented; b) improved permeability: as compared to hydroxy flavones, the methylated derivatives showed improved apical to basal transport in *in vitro* caco-2 cells; c) circumventing cytotoxic behaviour: while hydroxyl flavones had a tendency to undergo rapid metabolism *via* the involvement of myeloperoxidase, methylation of flavones appeared to prevent the metabolism involving that pathway. The lack of involvement of myeloperoxidase in turn cuts down the production of free radicals and eliminates any possibility of cytotoxic damage to the tissue(s) and/or cells; d) target specificity – potent inhibition of cytochrome P450 (CYP) 1B1: recent reports point out the involvement of CYP1B1 in smoking induced oral and oesophageal cancer and methoxylated flavones showed dramatic activity in reducing the mRNA expression of CYP1B1. In this regard, a few methoxylated flavones were confirmed to be potent inhibitors of CYP1B1 isozyme; e) retention of aromatase inhibition – one of the trade marks of hydroxy flavones was pertaining to the inhibitory property on aromatase enzyme especially useful in treatment and/or prevention of hormone-dependent cancer. In this context, the methoxylated flavones appeared to retain the aromatase inhibitory property showing similar potency to its hydroxyl counterparts. Although not explicitly confirmed with methoxylated flavonoids, other purported mechanisms generally attributed to this class of agents include: a) profound enzymatic enhancement of Phase II enzymes such as glutathione S-transferase and UDP-glucuronyl transferase to facilitate scavenging activities, b) facilitating the rampant formation of glutathione conjugates with the reactive agents/free radicals [9].

ANALOGY TO MODERN DAY DISCOVERY PROGRAM AND OTHER ADVANTAGES

The discovery of methylated flavones draws an apt parallelism to the drug discovery program in an industry setting – the key first steps in the pharmaceutical industry would entail creation of a chemical scaffold and/or a pharmacophore to bind/interact/modulate the given target with certain specificity and potency (i.e., essential for the creation of a hit molecule/series). While it is important to create a chemical skeleton as a first step, it is equally important to further optimize the structural requirements to impart druggable and biopharmaceutical properties such as high metabolic stability, improved permeability, good solubility, optimal distribution to lipids (i.e., cLogP) – all of these in turn translating *in vivo* to produce good oral bioavailability. The imparting of drug-like properties in these chemical structures will ensure that *in vivo* prediction will be in expectation with the already established *in vitro* properties.

Although dietary flavonoids failed to achieve adequate levels *in vivo* that was required to elicit the desired response due to the lack of drug like properties, a naturally occurring chemical skeleton of hydroxy flavones was readily available for further optimization – analogous to a fast-follower approach in the present day drug discovery scenario. Alongside this fast-follower opportunity that could be readily exploited,

a huge pile of human safety and tolerability profiles derived from the consumption of these dietary flavonoids have made this an attractive proposition for further research. Therefore, it was indeed creative that such a naturally occurring chemical structure was exploited to the fullest extent in the creation of methoxylated flavones which in turn manifested some distinctive and encouraging properties. Based on preliminary reports, methoxylated flavonoids have showed superior oral bioavailability in rodent models which in turn translated into better tissue distribution profile as compared to hydroxy flavonoid counterparts.

DEVELOPMENT CONSIDERATIONS

A) Importance of Tissue Distribution and Metabolic Assessment Studies

The recent work of Cai *et al.* (2007) suggested that tricrin was better represented in the tissues than apigenin following daily intake for 7 days of the two agents [18]. The accumulation and the longer residence in the tissue(s) were tied to the metabolic fate experienced by the two flavonoids. Both flavonoids underwent glucuronidation and sulfation reactions; however, apigenin appeared to undergo more rapid glucuronidation as compared to tricrin [18].

B) Structure Related Issues – Interference with Transporter Systems

Shin *et al.* (2006) demonstrated that flavonoid such as quercetin, upon co-administration, can produce significant pharmacokinetic interactions in terms of bioavailability and exposure for an important anti-cancer compound, tamoxifen [19]. It was postulated that quercetin, can interfere at the presystemic metabolism of tamoxifen and/or at the efflux p-glycoprotein transporter pathway. Since the exposure of both parent and 4-hydroxy tamoxifen was increased by quercetin, it appeared that inhibition of efflux transporters was the key mechanism for this interaction [19].

C) Structure Related Issues – Differential Data on Induction of Transporters

Whereas chrysin can induce the expression and activity of an important transporter system, UGT1A1, using *in vitro* HepG2 and Caco-2 cell lines, Smith *et al.* (2005) has demonstrated the inducibility of UGT1A1 in primary human hepatocyte cultures was only possible by increasing the dosing frequency and/or by controlling its metabolism [20]. Therefore, it was concluded that *in vivo* induction of UGT1A1 would be limited by both the bioavailability and/or metabolism of chrysin [20].

D) Structure Related Issues – Poor Bioavailability in Humans

Walle *et al.* (2001) have demonstrated that chrysin, in spite of high doses tested in humans subjects, did not reach adequate plasma levels to elicit its purported beneficial effects [21]. It was shown that the drug was subjected to: a) high presystemic metabolism – glucuronidation and sulfation was both rapid and extensive; b) rapid efflux of the metabolites through the efficient transporter systems back into the intestine [21].

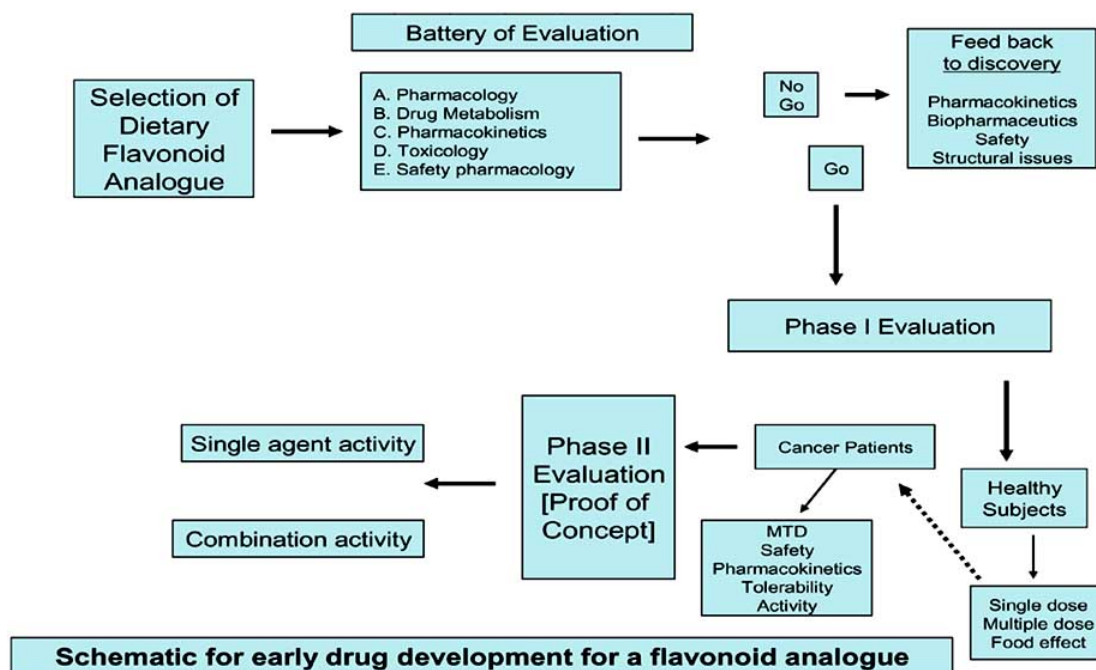


Fig. (1). Proposed development plan for the initial preclinical and clinical evaluation for a chosen flavonoid analogue.

E) Structure Related Issues – Specific Experience from Methylated Compounds

On the other side of the fence, there are some important lessons to be learnt from the use of methoxylated compounds such as the recent example of tramadol enantiomers [22]. Since polymorphic CYP2D6 was involved in the metabolism of tramadol the pharmacokinetic parameters were controlled by the phenotypic differences between the individuals (i.e., extensive and poor metabolizers) [22]. In this regard, possibility exists that methoxylated flavones could possibly undergo N-desmethylation reaction *in vivo* – this in turn may raise speculation for the possible involvement of CYP2D6 isozyme and surrounding complications of genetic polymorphism.

F) Brewing Controversy – Methylated Versus Non-Methylated Flavonoids

Landis-Piwowar *et al.* (2008) have proposed an interesting counter point on the continued labelling of methylated flavonoids as superior chemopreventive agents [23]. They claimed that blockade of 'free phenol' groups in devising novel methoxylated analogues would hinder the biological activity of the flavonoids. In a head to head study, it was shown that while methoxylated compounds had sustained levels and inhibited cell proliferation, it failed to show proteasome inhibitory property and was weakly apoptotic [23]. In contrast, unmethylated flavonoids exhibited multiple activities such as clear apoptotic signals through caspase activation, poly(ADP-ribose) polymerase cleavage and inhibition of proteasome [23]. Since it has been strongly concluded that methylated flavonoids may have limited ability to act as chemopreventive agents, the onus would be on the sponsor to ensure that such controversies are addressed during the early development stage of the chosen methylated flavonoid for drug development.

REGULATORY/CLINICAL STRATEGY AND OTHER CONSIDERATIONS

The unabated use of fruits, vegetables, herbal beverages etc to constantly supply the required dietary flavonoids cannot be expected from the development of pure substance(s) with a specific pharmacologic intent. It would possibly be a regulatory challenge to get an approval of a pure substance for the so-called chemopreventive properties in spite of the fact that such tall order claims are associated with the unregulated use of dietary consumables. Therefore, from a drug development perspective, it is important that an appropriate methoxylated flavone (mono methoxy or di-methoxy flavonoids and/or another novel structure) is picked for further pre-clinical testing to ensure that the pharmacological, pharmacokinetic, metabolic, tissue distribution and toxicokinetic profiles are well understood so that risk:benefit analysis would favour the clinical development of such class of compounds. Whereas, it is possible to dose healthy subjects to obtain some important safety, tolerability, pharmacokinetic and pharmacodynamic parameters, it is equally important to think of a regulatory and clinical strategy to enable its further development (i.e., how to go about establishing the activity in a clinical setting to convince the regulators of a probable indication for the marketability of such products). The possible use of these novel dietary flavonoids in the treatment of oral and oesophageal cancer, as suggested by others [24, 25], appears to be a prudent choice for clinical investigation; both as a single agent and/or in combination with other chemotherapeutics. Some evidences suggest that these agents may work in tandem with other known chemotherapeutic options, if deemed appropriate [26]. However, prior to charting out a clinical and regulatory path, it will be equally important to establish the single agent activity and as well as that of the combined entity in appropriate xenograft models for the chosen indication(s) – this would enable to put to-

gether a well defined IND package and development plan articulating both clinical and regulatory strategy (Fig. 1).

In this context, Syed *et al.* (2008) have provided some interesting thoughts on chemoprevention strategies for prostate cancer through the use of dietary supplements such as vitamins D/E, flavonoids, phytoestrogens, selenium related compounds, green tea polyphenols etc [27]. Some of the important issues that need to be tackled in assessing the clinical usefulness of such intervention therapy include: optimal period for dosing, route of administration, dosing frequency and optimization, gauge the toxicity of the agent(s), single agent approach and/or combination potential with other agents [27]. The work published by McClain and co-workers [28] provided some insights into how a preclinical safety and toxicology program could be developed for a naturally occurring compound such as genistein – a similar strategy with appropriate modifications may be readily applied for methylated flavonoids.

FUTURE DIRECTIONS

Various thoughts have been put forward for the development of methoxylated flavonoids in this note, by taking advantage of multi-mechanistic approaches exhibited by these agents, with careful and well planned clinical studies and appropriate supportive data. Additional, guidance from the regulatory agencies would greatly aid in streamlining the program and obtaining clarity on several lingering development issues. In order to appreciate, understand and resolve the issues, it is important to be abreast of other developments in the field. Nevertheless caution should prevail because the mechanisms that make flavonoids work in chemoprevention may render itself susceptible for numerous drug-drug interactions typically associated in the present day scenario of polypharmacy. This has been exemplified by numerous drug-drug interaction reports with other dietary consumables such as fruit juices [29] and/or herbal preparations such as St. John's wort [30]. Not but not the least, due to the importance of subject matter and increased participation of academic research occurring in this intriguing field of chemoprevention, one should be prepared to address any controversy that may surface from time to time and/or think of a proactive risk mitigation strategy if necessary.

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