Section 1: Overview

Motivation

Senolytics are agents that selectively induce apoptosis of senescent cells. Fisetin is a flavonoid polyphenol found in many types of fruits and vegetables (Ar ai et al., 2000) that is believed to act as a senolytic in addition to its numerous other known benefits. Although natural senolytics are less potent, compared to the targeted senolytics, they have lower toxicity and are thus, likely to be more readily translatable to clinical medicine. This RBA focuses on the risks and benefits of using fisetin as a senolytic rather than its more common use as a supplement.

Key Questions

This RBA seeks to answer the following questions:

- Which health and/or longevity benefits result from the use of fisetin as a senolytic?
- Which risks are involved in the use of fisetin as a senolytic (general and method-specific)?
- What are the potential risk mitigation strategies?
- Which method or combination of methods of using fisetin as a senolytic are most effective?
- Which of the available methods are safe for use?
- What is the best therapeutic protocol available at the moment?

Impatient readers may choose to skip directly to Section 6 for the conclusion and tips on practical application.

Section 2: Methods

Analytic model
The RBA has been prepared based on the principles outlined in *A Comprehensive Approach to Benefit-Risk Assessment in Drug Development* (Sarac et al., 2012).

**Literature search**

A literature search was conducted on Pubmed and the Cochrane Library using the search terms shown in Table 1. Titles and abstracts of the resulting studies were screened and relevant articles downloaded in full text. The references of the full-text articles were manually searched in order to identify additional trials that may have been missed by the search terms.

Because of the small number of papers available on the topic, we also searched for studies in which fisetin was found to influence any of the 6 known Senescence Cell Anti-Apoptotic Pathways (SCAPs).

**Table 1: Literature Search**

<table>
<thead>
<tr>
<th>Search terms</th>
<th>Number of publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>fisetin AND senolytic</td>
<td>5</td>
</tr>
<tr>
<td>fisetin AND aging</td>
<td>26</td>
</tr>
<tr>
<td>fisetin AND BCL</td>
<td>18</td>
</tr>
<tr>
<td>fisetin AND PI3 AND AKT</td>
<td>3</td>
</tr>
<tr>
<td>fisetin AND apoptosis</td>
<td>147</td>
</tr>
<tr>
<td>fisetin AND MDM2</td>
<td>1</td>
</tr>
<tr>
<td>fisetin AND p53</td>
<td>23</td>
</tr>
<tr>
<td>fisetin AND p21</td>
<td>7</td>
</tr>
<tr>
<td>fisetin AND serpine</td>
<td>5</td>
</tr>
<tr>
<td>fisetin AND HIF</td>
<td>4</td>
</tr>
<tr>
<td>fisetin AND toxicity</td>
<td>99</td>
</tr>
<tr>
<td>fisetin AND pharmacokinetic*</td>
<td>41</td>
</tr>
<tr>
<td>fisetin AND benefit*</td>
<td>23</td>
</tr>
<tr>
<td>fisetin AND risk*</td>
<td>23</td>
</tr>
</tbody>
</table>

**Other sources**

Discussion with experts (names cited in the text)

A manual search of the reference lists of the selected papers

**Recommended Reading**

The following sites offer information on fisetin as a senolytic at a consumer level and are useful as an introduction to the topic:

- Animal Data Shows Fisetin To Be A Surprisingly Effective Senolytic - fighthaging.org
- Fisetin May Be A Low Hanging Fruit For Aging - leafscience.org
- Fisetin: A Powerful Senolytic That Extends Health And Lifespan - alivebynature.com

**Abbreviation list**
MEF  |  murine embryonic fibroblasts  
HUVECs  |  human umbilical vein endothelial cells  
SCAPs  |  senescent cell antiapoptotic pathways  
SASP  |  senescence-associated secretory phenotype  
BW  |  bodyweight  
IMR90  |  human embryonic lung fibroblasts  
SA–gal  |  senescence-associated beta-galactosidase  
p16ink4a  |  a tumor suppressor protein  
p21Cip1  |  cell-cycle regulatory protein that interacts with cyclin-CDK2 and -CDK4, inhibiting cell cycle progression at G1  
MCP-1  |  monocyte chemotactic protein-1  

| Section 3: Existing evidence |

**Summary of ongoing clinical trials**

There are currently 3, phase 2 clinical trials underway (clinicaltrials.gov) and the first data is expected to be reported in about a year. The data from the phase 1 trials has not been published. All trials are being conducted by the same investigators at the Mayo clinic using the same treatment protocol.

<table>
<thead>
<tr>
<th>Institute</th>
<th>Date</th>
<th>Title</th>
<th>Participants</th>
<th>Dose</th>
<th>Duration</th>
<th>Main results</th>
<th>Primary Outcome Measures</th>
</tr>
</thead>
</table>
| Mayo Clinic  | Study Start Date: November 15, 2018  
Estimated Study Completion Date: April 28, 2020  | **Alleviation by Fisetin of Frailty, Inflammation, and Related Measures in Older Adults**  | Frail Elderly Syndrome Phase 2  
40; age 70-90; M and F  | 20 mg/kg/day, orally  
2 consecutive days  | No results reported yet  |  | percent decrease in blood inflammation markers (7 days)  |
| Mayo Clinic  | Study Start Date: February 6, 2018  
Estimated Study Completion Date: June 30, 2020  | **Alleviation by Fisetin of Frailty, Inflammation, and Related Measures in Older Women**  | Frail Elderly Syndrome Phase 2  
40; age 70-90; F  | 20 mg/kg/day, orally  
2 consecutive days, for 2 consecutive months.  | No results reported yet  |  | improved 6-minute walk (time frame: one month)  
i improved gait speed  |
| Mayo Clinic  | Actual Study Start Date: January 2, 2018  
Estimated Study Completion Date: April 2022  | **Inflammation and Stem Cells in Diabetic and Chronic Kidney Disease**  | Chronic Kidney Diseases; Diabetes Mellitus; Diabetic Nephropathies  
30; age 40-80; M and F  | 20 mg/kg/day, orally  
2 consecutive days  | No results reported yet  |  | change in inflammatory markers including C-reactive protein (14 days) in the skin, fat, plasma, and urine measured at baseline and day 14  
effect on mesenchymal stem cell function including cell migration measured at baseline and day 14  |

**Summary of results from preclinical trials (animals & in vitro)**

Only 2 papers directly related to the use of fisetin as a senolytic were identified, neither of which were conducted in humans (Yousefzadeh et al., 2018; Zhu et al., 2017). The other 5 studies included in the table relate to pharmacokinetics, risk, and lifespan extension.
<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Title</th>
<th>Species</th>
<th>Cell / Tissue type</th>
<th>Dose</th>
<th>Duration</th>
<th>Main results</th>
<th>Adverse effects</th>
<th>Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yousefzadeh et al.</td>
<td>2018</td>
<td>Fisetin is a senotherapeutic that extends health and lifespan</td>
<td>in vitro</td>
<td>MEF, IMR90</td>
<td>5 uM</td>
<td>48 h</td>
<td>• fisetin was the most effective flavonoid in reducing the fraction of SA–gal positive MEFs</td>
<td>none reported</td>
<td>• SA–gal activity</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>• fisetin reduced senescence in MEFs and IMR90 cells in a dose-dependent manner</td>
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<td>• p16INK4a expression</td>
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<td>• level of p16INK4a expression remained significantly lower in the fisetin-treated mice throughout the 4 wk period when the animals were not exposed</td>
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<td>• SASP markers</td>
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<td></td>
<td></td>
<td>• fisetin reduced expression of senescence and SASP markers significantly in all tissues (fat, spleen, liver, and kidney) and CD3+ T cells</td>
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<td>• IL-6, IL-8, MCP-1</td>
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<td></td>
<td>mice</td>
<td>progeroid mice</td>
<td>60 mg/kg /day intermittent exposure: from 6-8 wks and then 12-14 wks</td>
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<td>• significantly reduced the fraction of senescent cells in white adipose tissue</td>
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<td>• cell populations were mesenchymal stem/progenitor cells, T lymphocytes, natural killer cells, and endothelial cells</td>
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<td>• fisetin had no substantial effect on macrophages or dendritic cells</td>
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<td></td>
<td></td>
<td></td>
<td>human</td>
<td>ex vivo adipose tissue</td>
<td>20 uM</td>
<td>48 h</td>
<td>• significant reduction in SASP factors and SA–gal activity</td>
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<td></td>
<td>• selectivity reduced viability and numbers of HUVECs but not IMR90 or primary human preadipocytes</td>
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<td>• in HUVECs, fisetin induces apoptosis as measured by caspase 3/7 activity</td>
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<td>• in MEFs fisetin suppressed markers of senescence without evidence of cell killing</td>
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<td>• reduced viability at 0.5 uM</td>
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<td>• reduced viability at 5 uM</td>
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<td>• induced apoptosis at 0.5 uM</td>
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<td></td>
<td></td>
<td>• reduced cell numbers at 10 uM</td>
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</tbody>
</table>

| Zhu et al.     | 2017  | New agents that target senescent cells: the flavone, fisetin, and the BCL-X<sub>L</sub> inhibitor, A1331852 and A1155463 | in vitro                 | human preadipocytes, HUVECs, IMR90 | 0.5 uM, 5 uM, 10 uM | | | • selectively reduced viability and numbers of HUVECs but not IMR90 or primary human preadipocytes | n/a                           | • SA–gal activity        |
|                |       |                                                                       |                          |                    |          |          | • in HUVECs, fisetin induces apoptosis as measured by caspase 3/7 activity |                               | • SASP factor expression |
|                |       |                                                                       |                          |                    |          |          | • in MEFs fisetin suppressed markers of senescence without evidence of cell killing |                               | • IL-6, MCP-1            |
|                |       |                                                                       |                          |                    |          |          | • induced apoptosis at 0.5 uM |                               |                         |
|                |       |                                                                       |                          |                    |          |          | • reduced viability at 5 uM |                               |                         |
|                |       |                                                                       |                          |                    |          |          | • reduced cell numbers at 10 uM |                               |                         |
Demaria et al. 2014
An Essential Role for Senescent Cells in Optimal Wound Healing through Secretion of PDGF-AA

- senescent fibroblasts and endothelial cells appear very early in response to a cutaneous wound, where they accelerate wound closure by inducing myofibroblast differentiation through the secretion of platelet-derived growth factor AA (PDGF-AA)
- in two mouse models, topical treatment of senescence-free wounds with recombinant PDGF-AA rescued the delayed wound closure and lack of myofibroblast differentiation
- these findings define a beneficial role for the SASP in tissue repair and help to explain why the SASP evolved

Shia et al. 2009
Metabolism and Pharmacokinetics of 3,3,4,7-Tetrahydroxyflavone (Fisetin), 5-Hydroxyflavone, and 7-Hydroxyflavone and Anthemolysis Effects of Fisetin and Its Serum Metabolites

- after i.v. administration of fisetin (10 mg/kg of bw), fisetin declined rapidly and fisetin sulfates/glucuronides emerged
- when fisetin (50 mg/kg of bw) was given orally, fisetin was transiently present in serum only during the absorption phase, whereas fisetin sulfates/glucuronides predominated later
- serum metabolites of fisetin showed less potent inhibition on 2,2'-azobis(2-amidinopropane hydrochloride) (AAPH)-induced hemolysis than fisetin

Sengupta et al. 2004
Investigations on the binding and antioxidant properties of the plant flavonoid fisetin in model biomembranes

- fluorescence emission and excitation profiles along with fluorescence anisotropy data clearly indicate that fisetin molecules are localized and rigidly bound around the interfacial region (between the polar head and hydrophobic tail) of the egg PC liposomes
- this region is freely accessible to the approaching free radicals and serves as the reaction site for the antioxidant activity of the membranebound fisetin molecules
- experimental as well as theoretical studies on the antioxidant properties of fisetin indicate that this plant flavonoid has considerable potential for use as a therapeutic agent against free radicalmediated diseases

Sun et al. 2018
Anticancer effects of fisetin on mammary carcinoma cells via regulation of the PI3K/Akt/mTOR pathway: In vitro and in vivo studies

- fisetin downregulated p-PI3K, p-Akt and p-mTOR
- Bax and Bcl were associated with the fisetin-induced apoptosis of 4T1 cells
- fisetin + vehicle and vehicle alone were associated with an elevation of ALT+AST and ALT respectively, suggesting liver toxicity
- fisetin may aggravate liver burden because of its poor bioavailability
Section 4: Risk-Benefit Analysis

Decision Model

Risk and benefit criteria

The decision profile is made up of risk and benefit criteria extracted from the outcomes of the above-mentioned papers. The benefit criteria are organized by category and include the type, magnitude, and duration of the benefit as well as its perceived importance to the patient. The risk criteria are organized by category, type, severity, frequency, detectability, and mitigation. All are assigned numerical values:

1 = low
2 = moderate
3 = high

The numerical values for both risk and benefit criteria are then summarized serving as the justification for the weighting in the following column.

Weight

The criteria are weighted on a value scale to enable comparison (based on the relative importance of a difference). Risk and benefit criteria are assigned to either low (1-1.66), medium (1.67-2.33), or high (2.34-3) weighted categories.

Weighting is independent of data sets and the final weights are based on consensus with justification based on the preceding columns of the table.

Score

Each category is assessed according to the performance of fisetin therapy against the comparator (physiological aging) whereby a numerical value is assigned for each criterion -1 (inferior), 0 (equivalent or non-inferior) and +1 (superior) to the comparator.

Uncertainty

Uncertainty is determined according to the amount and quality of the evidence, whether it came from human or animal studies and whether methodological flaws, conflicting studies, or conflicts of interest (funding) by the authors are present. Human evidence is initially assigned a score of "1", evidence from rodent studies, "2", and in vitro or lower animal studies, "3". The uncertainty score is then adjusted by upgrading or downgrading using the above-mentioned criteria.

Weighted score

The weights and scores are multiplied to produce weighted scores that enable direct comparison (-3 +3) and then adjusted using the uncertainty score. Weighted scores may be upgraded where the uncertainty of the evidence is low or downgraded where the uncertainty of the evidence is high.

Benefit assessment

Table 4: Benefit assessment
## Benefits

### Senescent cells

Accumulation of senescent cells is one of the major phenomena underlying aging and chronic disease. Senescent cells are resistant to apoptosis by virtue of 6 known senescent cell antiapoptotic pathways (SCAPs). SCAPs allow the survival of senescent cells despite the secretion of the proapoptotic SASP. THe SCAPs required for senescent cell resistance to apoptosis vary according to cell type.

Agents targeting a single SCAP may not eliminate all types of senescent cells and so far, all senolytics that have been tested have exhibited a certain degree of cell-type specificity. Fisetin has been found to interfere with 4 of the 6 known SCAPs in several in vitro studies (Pal et al., 2013; Zhu et al., 2017; Zhang et al., 2016; Li et al., 2011; Triantafyllou et al., 2008; Min et al., 2017; Sabarwal et al., 2017) leading to interest in its use as a senolytic agent. Fisetin was shown to inhibit BCL-2 (an inhibitor of apoptosis) (Verma et al., 2017; Shih et al., 2017; Tsai et al., 2019) by binding to a hydrophobic groove on the protein (Verma et al., 2017). It has also been shown to induce apoptosis through ROS production (Sabarwal et al., 2017) and increased activity of caspase-3, -8 and -9 (Tsai et al., 2019). Fisetin also increased apoptosis via p53 mediated up-regulation of DR5 expression at the transcriptional level (Min et al., 2017).

<table>
<thead>
<tr>
<th>Category</th>
<th>Subjects</th>
<th>Benefit type</th>
<th>Magnitude</th>
<th>Likelihood</th>
<th>Duration</th>
<th>Importance to patient</th>
<th>Summary</th>
<th>Weight</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Senescent cells</td>
<td>mice levels of p16Ink4a expression</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>2.25</td>
<td>+1</td>
</tr>
<tr>
<td>2</td>
<td>Senescent cells</td>
<td>MEF culture, ex vivo human adipose tissue</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>10</td>
<td>2.5</td>
<td>+1</td>
</tr>
<tr>
<td>3</td>
<td>Senescent cells</td>
<td>aged mice, ex vivo human adipose tissue</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>2.25</td>
<td>+1</td>
</tr>
<tr>
<td>4</td>
<td>Senescent cells</td>
<td>naturally aged 100 mg/kg/day, orally reduced the fraction of senescent cells in white adipose tissue (stem/progenitor cells, T lymphocytes, natural killer, and endothelial cells)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>2.25</td>
<td>+1</td>
</tr>
<tr>
<td>5</td>
<td>Health/life span</td>
<td>aged mice + flies median and maximal lifespan</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>2.25</td>
<td>+1</td>
</tr>
<tr>
<td>6</td>
<td>Metabolism</td>
<td>aged mice amylase</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>1.75</td>
<td>+1</td>
</tr>
<tr>
<td>7</td>
<td>Metabolism</td>
<td>aged mice ALT</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>1.75</td>
<td>+1</td>
</tr>
<tr>
<td>8</td>
<td>Metabolism</td>
<td>aged mice oxidative stress in the liver</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>+1</td>
</tr>
<tr>
<td>9</td>
<td>Senescent cells</td>
<td>in vitro HUVECs apoptosis (endothelial)</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>2.25</td>
<td>+1</td>
</tr>
<tr>
<td>10</td>
<td>Senescent cells</td>
<td>in vitro HUVECs cell viability (endothelial)</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>1.75</td>
<td>+1</td>
</tr>
<tr>
<td>11</td>
<td>Senescent cells</td>
<td>in vitro HUVECs cell numbers (endothelial)</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td>2.25</td>
<td>+1</td>
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</tbody>
</table>
In studies directly examining its effects on senescence, fisetin has been shown to significantly reduce markers of senescence such as p16Ink4a expression, SA--gal activity/expression, and SASP markers in fat, spleen, liver and CD3+ T cells in mice as well as ex vivo, in human adipose tissue (Yousefzadeh et al., 2018). It has also been shown to reduce the fraction of senescent cells (T-cells, stem cells, natural killer cells, and endothelial cells but not macrophages or dendritic cells) in white adipose tissue of naturally aged mice (Yousefzadeh et al., 2018).

In vitro, fisetin was found to dose-dependently increase apoptosis, reduce cell viability, and reduce senescent cell number in HUVECs but not IMR90 (fibroblasts) or primary human preadipocytes indicating a degree of cell-type specificity.

**Metabolism**

Positive effects on pancreatic and liver homeostasis were also seen in significantly lower levels of ALT and amylase and decreased oxidative stress in the liver (Yousefzadeh et al., 2018).

**Lifespan**

Fisetin administration beginning at 85 weeks of age (approximately equivalent to human age 75) was shown to increase lifespan in mice by about 10% (Yousefzadeh et al., 2018). In vitro, fisetin 10 µm did not change the lifespan of flies but 100 µm fisetin increased lifespan by up to 23% (Wood et al., 2004).

**Risk assessment**

<table>
<thead>
<tr>
<th>Table 5: Risk assessment</th>
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</thead>
<tbody>
<tr>
<td>Category</td>
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<tr>
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</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>3</td>
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</tbody>
</table>

**Individual risks**

Very little is known about the potential side effects of senolytic drugs as a class. A study of genetic clearance of senolytic cells has shown a delay in wound healing and increased fibrosis after the wound is healed (Demaria et al., 2014). A second study shows that senescent cells function to limit fibrosis during liver regeneration and that impairment of this function leads to increased fibrosis (Krizhanovsky et al., 2008). A third, purely hypothetical risk is cell lysis syndrome due to the sudden death of many cells. This is, however, highly unlikely because even in aged tissue, the proportion of senescent cells is about 15% (Herbig et al., 2006) and senolysis has been shown to lead to a reduction of about 30-40% of senolytic cells (Zhu et al., 2015). These risks are relatively easy to mitigate through intermittent dosing that is limited to periods of good health.

Only one animal study on fisetin has reported any form of toxicity from fisetin use and the authors concluded that the elevations in ALT/AST levels (indications of liver toxicity) were in large part due to the vehicle used to administer the fisetin (DMSO). However, the fisetin + vehicle group showed significantly higher elevations than the vehicle alone group indicating that high doses of fisetin may additionally burden the liver because of its poor bioavailability (Sun et al., 2018). At a lower dose (112 mg/kg), fisetin didn't significantly increase apoptosis or lead to liver toxicity (Sun et al., 2018). Intermittent dosing and use of a form of fisetin with increased bioavailability are likely to mitigate the risk of liver toxicity.

**Summary of the evidence on pharmacokinetics, form, dose, and duration**

**Pharmacokinetics**

To the best of our knowledge, there haven't been any studies published on the pharmacokinetics of fisetin in humans.
Fisetin is a potent bioactive compound with limited bioavailability because of its low aqueous solubility and poor absorption from the gut. The absolute bioavailability of fisetin was calculated as 7.8% and 31.7% after oral administration of 100 and 200 mg/kg fisetin, respectively (Jo et al., 2017). Fisetin concentrations achieved in a mouse study without toxicity (2.7-349.4 µM) (Touil et al., 2011) are higher than those found to be senolytic in vitro (Zhu et al., 2017). The biological activity of fisetin depends on the presence of hydroxyl groups at 3, 7, 3, 4 positions and the oxo group at position 4 with a double bond between C2 and C3 (Kashjap et al., 2018). The hydroxyl group at C-7 and the double bond between C2 and C3 are essential for its antioxidant activity (Sengupta et al., 2004).

Several studies have reported that the solubility and bioavailability of fisetin can be improved by cocrystallization with caffeine, isonicotinamide, and nicotinamide, complexation with cyclodextrins and encapsulation with nanoparticles (Kashjap et al., 2018). Fisetin micelles demonstrate a more sustained and prolonged in vitro release behavior, as well as enhanced cytotoxicity, cellular uptake, and fisetin-induced apoptosis (Chen et al., 2015).

The metabolism of fisetin was first determined in rats following intravenous and oral administration (Shia et al., 2009). Flavonoids are known to be extensively metabolized following oral consumption resulting in glucuronidated, sulfated and methylated metabolites. Following intravenous injection in rats (10 mg/kg), blood levels of fisetin rapidly declined with the appearance of sulfate and glucuronide-containing conjugates. Following oral administration (50 mg/kg), fisetin was only transiently present in rat serum and was replaced by conjugated sulfates and glucuronides. The serum concentration of fisetin sulfates/glucuronides was maintained at 10 µM for >24 h (Maher, 2017). The half-life of fisetin in female mice (3.12 h) following i.v. administration was longer than in male mice (Kashjap et al., 2018).

Significant accumulation of fisetin and its metabolites in multiple tissues was also seen. The main metabolites were glucuronidated fisetin, geraldol (3,4,7-trihydroxy-3-methoxyflavone) and glucuronidated geraldol. Geraldol was as active as fisetin in several biological assays.

In macaques fed a single oral dose of 25 mg/kg bw, it was found that sulfated and/or glucuronidated forms of fisetin reach concentrations of 30 µM in the cerebrospinal fluid with a plasma half-life of 8 h (Maher, 2017).

Due to the commercial unavailability of most conjugates, only a few studies have been performed on the biological effects of fisetin metabolites. Results varied showing, on one hand, significantly lower activity against erythrocyte hemolysis for sulfates/glucuronides (Shia et al., 2009) but somewhat stronger cytotoxic activity on lung carcinoma cells as compared to intact fisetin. Geraldol could also inhibit endothelial cell migration and proliferation. Combined with fisetin's rapid half-life of 9 minutes this suggests that fisetin metabolites play an important role in its in vivo activities (Touil et al., 2011).

Form

Fisetin has been administered by i.v. and orally in animals. Current clinical trials use oral supplements and many investigations are being carried on the theme of increasing its bioavailability. Suppositories have also been suggested as a means of increasing bioavailability and are the method of choice of some well-known health care professionals (Joseph Mercola). However, there are no studies that have measured the amount of fisetin absorbed rectally in humans.

Dose

The clinical trials currently in progress use a dose of 20 mg/kg bw, administered on two consecutive days (Table 2).

The pharmacologic activity of fisetin has been shown to vary according to the concentration achieved within the cells. Similar to quercetin, fisetin, showed protective effects against H2O2-induced cytotoxicity, DNA strand breaks, and apoptosis at concentrations as low as 10–25 µmol/L. On the other hand, these flavonoids induced cytotoxicity, DNA strand breaks, oligonucleosomal DNA fragmentation, and caspase activation at concentrations between 50 and 250 µmol/L. Data suggests that cytoprotective concentrations of some flavonoids are lower by a factor of 5–10 than their DNA-damaging and proapoptotic concentrations (Waetjen et al., 2005).

Duration

Although yet to be confirmed for fisetin, intermittent administration of some senolytic compounds has been shown to be adequate (Zhu et al., 2015). Since a brief disruption of pro-survival pathways is enough to kill senescent cells, senolytics don’t have to be present continuously to be effective (Zhu et al., 2015). The frequency of senolytic treatment will depend on rates of senescent cell reaccumulation, which probably varies according to conditions (Kirkland et al., 2017). Advantages of intermittent administration include less chance of developing adverse and off-target effects and the ability to choose periods of good health for administration.

Toxicity

There is no evidence at the present time for either short- or long-term toxicity from fisetin supplementation. Mice that were orally administered fisetin at 2000 mg/kg bodyweight, examined for 48 h and then sacrificed showed no indication of toxicity (Maher, 2017). The effects of long-term feeding of fisetin (25 mg/kg bodyweight over nine months) have also been evaluated in mice. No significant difference in body weight was seen in animals supplemented with fisetin. Additionally, multiple tissues (lungs, spleen, liver, kidneys, heart, stomach, intestine, testes and ovary) were examined using standard toxicological criteria and no toxicity was found. Furthermore, fisetin has no inhibitory effects on the activities of cytochrome P450s 3A4, 2C9 and 2D6 at concentrations up to 10 µM (Maher, 2017).

The LD50 of fisetin in rats by i.v. is 180 mg/kg (www.caymanchem.com).

Drug Interactions
There is a lack of information on drug interactions with fisetin. As fisetin has been shown to lower blood glucose in diabetic rats (given at 10 mg/kg bodyweight for 30 days) (Prasath et al., 2014) it is conceivable that it could potentiate the effects of glucose-lowering drugs such as metformin. This risk is likely lessened by the intermittent dosing schedule.

Section 5: Presentation of Results

The following "tornado" diagram summarizes the results of the previous sections:

- The risk-benefit criteria are listed in the category column.
- The weighted score after factoring in uncertainty is shown as a numerical value.
- The weight of the criteria is proportional to the width of the columns.
- Risk and benefit criteria are assigned to either low (1-1.66), medium (1.67-2.33), or high (2.34-3) weighted categories based on the results of the assessment in Table 4 and Table 5.
- The diagram is filterable by category so the main risks and benefits for each system can be viewed.

To view the tornado diagram as a pdf please click on the thumbnail below:

![Tornado Diagram](Fisetin RBA v1.png)

For those who would prefer to view the document in excel, we have included the original .xls file.

*Fisetin RBA v1.xlsx*

Section 6: Conclusions of the Risk-Benefit Analysis

**Main benefits**

Fisetin has been shown to decrease senescent cell biomarkers as well as the numbers of senescent cells in a variety of tissues, including *ex vivo*, human adipose tissue as well as *in vivo*, in mice.

**Main risks**

Reduction of senescent cells has been associated with delayed wound healing and an increased level of fibrosis after healing (Demaria et al., 2014). Fisetin was associated with liver toxicity in mice at doses of 223 mg/kg administered over 3 weeks (Sun et al., 2018). There is also the possibility of drug interactions with glucose-lowering drugs such as metformin.
Risk Mitigation Strategies

- Wait to commence therapy until clinical trial data has been published that describes the possible benefits and adverse effects
- Consult your physician before beginning therapy
- Frequency of therapy is to be determined by the results of the senescent cell biomarker lab panel
- Measure baseline senescent cell load biomarkers including SA-β-gal activity, p16Ink4a expression, p21Cip1 expression, and SASP factors: IL-6, IL-8, MCP-1 when possible
- Repeat screening at regular intervals to determine the effectiveness of the therapy, and the appropriateness of the dose
- Measure blood values, liver & kidney function and electrolyte values at regular intervals
- Use caution when combining fisetin with glucose-lowering drugs (monitor blood sugar levels)
- Avoid or use with extreme caution if nonalcoholic fatty liver disease has been diagnosed or is suspected
- Cease therapy if any identifiable adverse effects occur

Section 7: Practical Application

Form & Dose

- Follow the risk mitigation strategies outlined in Section 6
- At the current time, the only form and dose that has been tested in phase 1 clinical trials is the so-called “Mayo Protocol”
- The Mayo Protocol consists of taking 20 mg/kg body weight of oral fisetin on two consecutive days and repeating the same dose, one month later

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