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Review article

Repurposing digoxin for geroprotection in patients with frailty and multimorbidity

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ABSTRACT

The geroscience hypothesis proposes biological hallmarks of ageing are modifiable. Increasing evidence supports targeting these hallmarks with therapeutics could prevent and ameliorate age-related conditions – collectively termed "geroprotector drugs". Cellular senescence is a hallmark with considerable potential to be modified with geroprotector drugs. Senotherapeutics are drugs that target cellular senescence for therapeutic benefit. Repurposing commonly used medications with secondary geroprotector properties is a strategy of interest to promote incorporation of geroprotector drugs into clinical practice. One candidate is the cardiac glycoside digoxin. Evidence in mouse models of pulmonary fibrosis, Alzheimer's disease, arthritis and atherosclerosis support digoxin as a senotherapeutic agent. Proposed senolytic mechanisms are upregulation of intrinsic apoptotic pathways and promoting intracellular acidification. Digoxin also appears to have a senomorphic mechanism - altering the T cell pool to ameliorate pro-inflammatory SASP. Despite being widely prescribed to treat atrial fibrillation and heart failure, often in multimorbid older adults, it is not known whether digoxin exerts senotherapeutic effects in humans. Further cellular and animal studies, and ultimately clinical trials with participation of pre-frail older adults, are required to identify whether digoxin has senotherapeutic effect at low dose. This paper reviews the biological mechanisms identified in preliminary cellular and animal studies that support repurposing digoxin as a geroprotector in patients with frailty and multimorbidity.

1. Introduction

An increasing proportion of older adults live with multimorbidity, the presence of two or more chronic conditions (Johnston et al., 2018). Multimorbidity contributes to increased disability, frailty, and economic burden (Johnston et al., 2018; Marengoni et al., 2011; Nunes et al., 2016; Soley-Bori et al., 2021; Wang et al., 2020). By 2050 25% of the UK population are projected to be over 65 years old with over half of the 65- to 74-year-old cohort being multimorbid (Kingston et al., 2018; ONS, 2021; Salomon et al., 2012; UN, 2013). Multimorbidity can create discrepancy between an individual's health-span (the number of years spent in good health) and total lifespan (Crimmins, 2015). Current medical management of multimorbidity relies on polypharmacy, as individual medication(s) are prescribed for specific diseases (Ermogenous et al., 2020). Polypharmacy is associated with risk of hospitalisation and frailty, but this may in part be due to iatrogenic drug-drug/drug disease interactions (Fried et al., 2014; Gutiérrez-Valencia et al., 2018; Leelakanok et al., 2017; Maher et al., 2014; Wastesson et al., 2018).

Multimorbidity is now recognised as the phenotype of advanced maladaptive biological ageing and not just the coincidental presence of multiple chronic diseases (Fabbri et al., 2015; Fraser et al., 2022; Prados-Torres et al., 2014). Cellular damage underlies age related conditions, and is driven by fourteen "hallmarks" of ageing (Schmauck--Medina et al., 2022). These comprise nine hallmarks first proposed by Lopez-Otin et al. in 2013 – genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, altered nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (Lopez-Otin et al., 2013) – plus five hallmarks recently added by Schmauck-Medina et al. – compromised autophagy, dysregulation of RNA processing, microbiome disturbance, altered mechanical properties of cells and extracellular milieu, and inflammation (Schmauck-Medina et al., 2022). Research

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increasingly supports targeting these hallmarks at an early stage to modify the development of age-related disease and multimorbidity – a concept known as the geroscience hypothesis (Kennedy et al., 2014).

Lifestyle interventions (such as physical activity, healthy diet, weight loss, home modifications to improve mobility and functioning) should be encouraged as part of a holistic management approach to older adults (Erusalimsky, 2020; Rimland et al., 2016). However, implementing these in the real world may not always be feasible or acceptable due to reasons such as physical limitations, social or cultural differences. For many adults, lifestyle interventions alone are not enough to ameliorate development of age-related conditions (Partridge et al., 2018). Medication is therefore likely to continue being an important tool. The proposed role of medication within the geroscience hypothesis is to target hallmarks of ageing – collectively termed geroprotector drugs (Moskalev et al., 2017; Trendelenburg et al., 2019). Clinical trials are required to determine optimal time to give geroprotector drugs within the adult lifespan, however the consensus is they would be given in older healthy individuals before onset of age-related disease, multimorbidity, or frailty (Aliper et al., 2016; Konopka and Miller, 2019).

2. Cell senescence

Cellular senescence is a hallmark of ageing with considerable potential to be modified with geroprotetctor drugs (Partridge et al., 2020). Senescence is a state where cells enter stable cell cycle arrest (Gorgoulis et al., 2019; Hernandez-Segura et al., 2018) and therefore provides an important mechanism to prevent cancer – the unregulated propagation of damaged cells (Hanahan and Weinberg, 2000). Senescent cells accumulate with age and exposure to mutagenic triggers such as DNA damage, ultraviolet radiation, oxidative stress and drug toxicity (Gorgoulis et al., 2019; Lopez-Otin et al., 2013). Broadly, senescent cells are characterised in vitro by a flat morphology with high lysosome activity reflected by high levels of senescence-associated beta galactosidase lysosomal enzyme (SA-β-Gal) (Dimri et al., 1995; Gorgoulis et al., 2019; Hernandez-Segura et al., 2018). Senescent cells accumulate cyclin-dependent kinase inhibitors $p21^{WAF1/Cip1}$ (CDKN1A) and $p16^{INK4A}$ (CDKN2A), preventing the phosphorylation of pRB required for cell-cycle progression (Rodier and Campisi, 2011; Sharpless and Sherr, 2015).

Despite being in cell cycle arrest, senescent cells release a cocktail of pro-inflammatory chemicals known as the Senescent Associated Secretory Phenotype (SASP) (Coppé et al., 2010; Gorgoulis et al., 2019; Hernandez-Segura et al., 2018; Rodier and Campisi, 2011). The SASP comprises cytokines, chemokines, matrix metalloproteinases, and growth factors which in the short-term promote beneficial immune responses, aid cell repair and wound healing (Coppé et al., 2010; Gorgoulis et al., 2019). However chronic release contributes to tissue inflammation which contributes to the development of age-related conditions

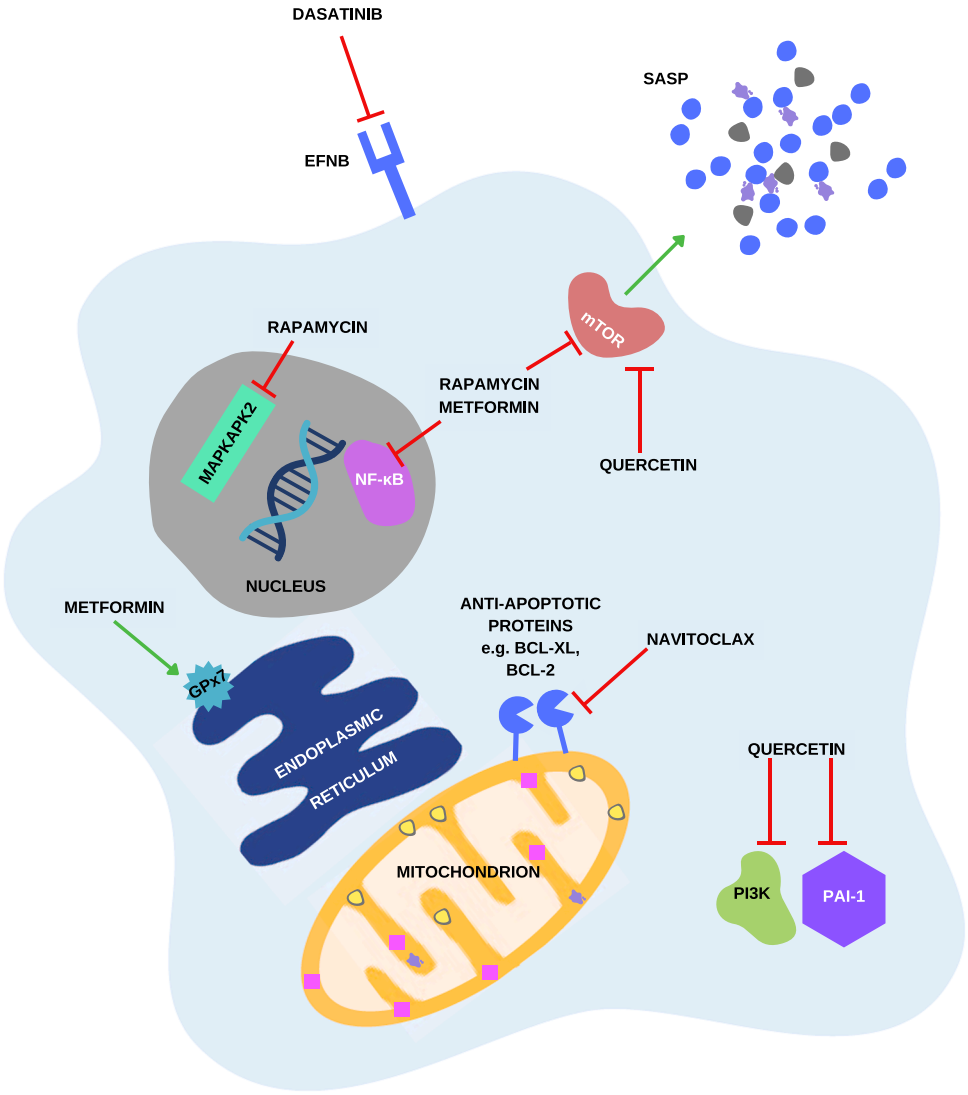

Fig. 1. Figure 1: Senotherapeutic mechanisms. BCL-2 B-cell lymphoma 2; EFNB ephrin receptor tyrosine kinase; GPx7 glutathione peroxidase; MAPKAPK2 mitogen-activated protein kinase-activated protein kinase 2; mTOR mammalian Target of Rapamycin; NF-κB nuclear factor of kappa light polypeptide gene enhancer in B-cell; PAI-1 plasminogen-activator inhibitor 1; PI3K Phosphoinositide 3-kinase. Senolytics selectively induce apoptotic clearance of senescent cells. Examples of these are dasatinib which inhibits EFNB activity; quercetin which inhibits pro-survival mTOR/PI3K pathways and PAI-1; and navitoclax which inhibits mitochondrial anti-apoptotic BCL-2 proteins. Senomorphics attenuate pro-ageing and inflammatory effects of the SASP without clearing senescent cells. Examples of these include rapamycin which inhibits mTOR, suppresses NF-κB transcription factor and MAPKAPK2; and metformin which also inhibits mTOR, suppresses NF-κB and upregulates endoplasmic reticulum-localized GPx7.

(Ferrucci and Fabbri, 2018; Franceschi et al., 2000).

3. Senotherapeutic mechanisms

Senotherapeutics are drugs which target senescent cells for therapeutic benefit (Niedernhofer and Robbins, 2018; Zhang et al., 2022). They are divided into senolytics, those that selectively induce apoptotic clearance of senescent cells, and senomorphics, which attenuate pro-ageing and inflammatory effects of the SASP without clearing senescent cells, (Niedernhofer and Robbins, 2018; Zhang et al., 2022) Fig. 1.

3.1. Senolytics

The first senolytics were discovered by Zhu : dasatinib, a tyrosine-kinase inhibitor used to treat haematological malignancies, and quercetin, a plant flavonoid (Zhu et al., 2015). Dasatinib inhibits EFNB (ephrin receptor tyrosine kinase) activity, a pro-survival dependence receptor implicated in tumorigenesis (Goldschneider and Mehlen, 2010; Xi et al., 2012). Quercetin inhibits pro-survival mammalian Target of Rapamycin/Phosphoinositide 3-kinase (mTOR/PI3K) pathways (Bruning, 2013) and plasminogen-activator inhibitor 1 (PAI-1) Fig. 1 whose overexpression contributes to development of cardiovascular disease (Olave et al., 2010). Zhu demonstrated dasatinib and quercetin individually induced clearance in several senescent human cell lines (human preadipocytes and human umbilical vein cells, HUVECs) but combination treatment with both agents had greatest senolytic effect (Zhu et al., 2015). In old mice, combination treatment using a “hit and run approach” – high dose treatment over a short duration - improved exercise capacity and left ventricular ejection fraction (Zhu et al., 2015). Other aged mouse studies showed that biweekly combination treatment extended lifespan (Xu et al., 2018).

Zhu et al. also identified that the B-cell lymphoma 2 (BCL-2) inhibitor drug class had senolytic properties (Zhu et al., 2016). These agents inhibit anti-apoptotic BCL-2 proteins, disrupting integrity of the mitochondrial outer membrane and facilitates protease-mediated cell destruction (Kale et al., 2018; Vogler et al., 2009) Fig. 1. Navitoclax (a BCL-2 inhibitor), used to treat haematological malignancies and small-cell lung cancer (Billard, 2013; Vogler et al., 2009; Wendt, 2008), induced senolysis of HUVECs, IMR90 human lung fibroblasts, and murine embryonic fibroblasts (Zhu et al., 2016). In mouse models, navitoclax improved haematopoetic stem cell function through clearance of senescent bone marrow cells (Chang et al., 2016). Mice treated with navitoclax prior to induction of myocardial infarction had attenuated decline in ejection fraction and improved survival (Walaszczyk et al., 2019).

A small number of clinical trials have assessed senolytics in humans. Martyanov et al. were the first and assessed senolytic potential of dasatinib in 31 participants with pulmonary fibrosis over a nine month period, demonstrating amelioration of radiological disease progression (Martyanov et al., 2017). Justice et al. studied the effect of intermittent dasatinib and quercetin treatment in 14 participants with pulmonary fibrosis over three weeks (total of 9 dosing days over three week period) (Justice et al., 2019). Statistically significant and clinically meaningful improvements in 6-min walk distance, 4-m gait speed, and chair-stands time were observed one week after completion of the dosing schedule (Justice et al., 2019). Using a “hit-and-run approach”, dasatinib and quercetin treatment for 3 days showed reduced senescent cell burden within adipose and skin tissue (Hickson et al., 2019).

Effectiveness of senolytics in specific human disease cases is under current intensive study but the long-term effects are not known, nor are the effects on relatively healthy individuals. Potential side effects of dasatinib and BCL-2 inhibitors of myelosuppression and pancytopaenia may deter their use. However, safety data from clinical trials for senolytic effect are reassuring - mild diarrhoea and nausea were the most common adverse events with dasatinib (Hickson et al., 2019; Justice et al., 2019; Martyanov et al., 2017), consistent with large randomised controlled trial data (Richeldi et al., 2014), and only a small number of serious adverse events were reported (oedema, dyspnoea and pulmonary fibrosis)(Justice et al., 2019; Martyanov et al., 2017). Further research is required before senolytics could be introduced in clinical practice. Over sixty clinical trials are ongoing to study effects of senolytic agents in older adults including those with Alzheimer’s disease (Clinicaltrials.gov, 2022a), osteoporosis (Clinicaltrials.gov, 2021b), chronic kidney disease (Clinicaltrials.gov, 2021a), and nursing home residents with COVID-19 (Clinicaltrials.gov, 2022b). Full lists of current and ongoing clinical trials have recently been published (Gasek et al., 2021; Raffaele and Vinciguerra, 2022).

3.2. Senomorphics

A number of drugs already in clinical use have secondary senomorphic properties - two of the most researched drugs are rapamycin and metformin. Rapamycin, an inhibitor of pro-anabolic protein kinase mTOR, is an immunosuppressant used to prevent acute kidney allograft rejection (Hahn et al., 2019; Liu and Sabatini, 2020). SASP expression is mTOR dependent (Herranz et al., 2015; Laberge et al., 2015), and rapamycin suppresses master regulators of SASP expression NF-κB transcription factor (Nuclear Factor of kappa light polypeptide gene enhancer in B-cells transcription factor) (Laberge et al., 2015) and mitogen-activated protein kinase-activated protein kinase 2 (Herranz et al., 2015) Fig. 1.

Several studies demonstrate rapamycin suppresses SASP in human, mice, and rat cell lines (Selvarani et al., 2021). Rapamycin is a senomorphic of particular interest by demonstrating consistent extension of lifespan in genetically heterogenous mouse models (Flynn et al., 2013; Harrison et al., 2009; Miller et al., 2010, 2014; Nadon et al., 2017). Rapamycin also prolongs health-span in mouse models of cardiac dysfunction (Flynn et al., 2013) and cognitive impairment (Majumder et al., 2012; Ozcelik et al., 2013; Spilman et al., 2010). Two clinical trials in older adults showed the rapamycin analogue Everolimus boosted immune responses to the influenza vaccine and reduced rates of respiratory tract infection, showing greatest effect in the oldest old participants and those with asthma and diabetes (Mannick et al., 2014, 2018). Gastrointestinal symptoms and mouth ulcers were most common adverse events with only one serious adverse event attributed to the drug (severe mouth ulcers)(Mannick et al., 2014).

Metformin, a biguanide antidiabetic drug, also inhibits the mTOR pathway (Barzilai et al., 2016; Cabreiro et al., 2013; Martin-Montalvo et al., 2013) but the mechanism by which it suppresses SASP expression is not fully understood. Proposed mechanisms include NF-κB inhibition (Moiseeva et al., 2013), upregulation of endoplasmic reticulum-localized glutathione peroxidase 7 (Fang et al., 2018), and brain-derived neurotrophic factor (Śmieszek et al., 2017) Fig. 1.

Metformin extends health-span in aged mice, demonstrating improved physical performance and cognitive function (Allard et al., 2016) as well as extending lifespan (Anisimov et al., 2008). To date research on metformin in human health has tended to focus on diabetes and cardiovascular disease (Goldberg et al., 2015, 2013; Knowler et al., 2002; UKPDS, 1998) rather than its potential to ameliorate underlying processes of unhealthy ageing. A small number of studies have assessed the effects of metformin in older adults after physical activity which demonstrated unfavourable results of blunted mitochondrial respiration and muscle hypertrophy (Konopka et al., 2019; Peterson et al., 2018). In contrast, the planned Targeting Ageing with Metformin (TAME) trial is a U.S. multi-centre randomised controlled trial which will assess the effect of metformin on ageing more holistically (Barzilai et al., 2016; Justice et al., 2018). Rather than measure single end-points of individual disease, the trial will assess efficacy on broad outcomes: a) death or any new age related chronic disease, b) functional outcomes - major decline in mobility or cognitive function, or onset of activities of daily living limitation (Justice et al., 2018).

4. Evidence of digoxin as a senotherapeutic agent

Digoxin is a cardiac glycoside derived from *Digitalis purpura* (Withering, 2014). Digoxin is routinely used at low-dose in the management of atrial fibrillation (AF) and heart failure, particularly in older adults with multi-morbidity (Hindricks et al., 2020; McDonagh et al., 2021; Ziff and Kotecha, 2016). Although digoxin does not impact on mortality in these patients, digoxin reduces heart rate with a significant reduction in hospital admissions (Kotecha et al., 2020; Ziff et al., 2015). The main pharmacological site of action is the sodium-potassium-adenosine triphosphate pump, 3Na-2 K-ATPase (Schoner and Scheiner-Bobis, 2007) Fig. 2.

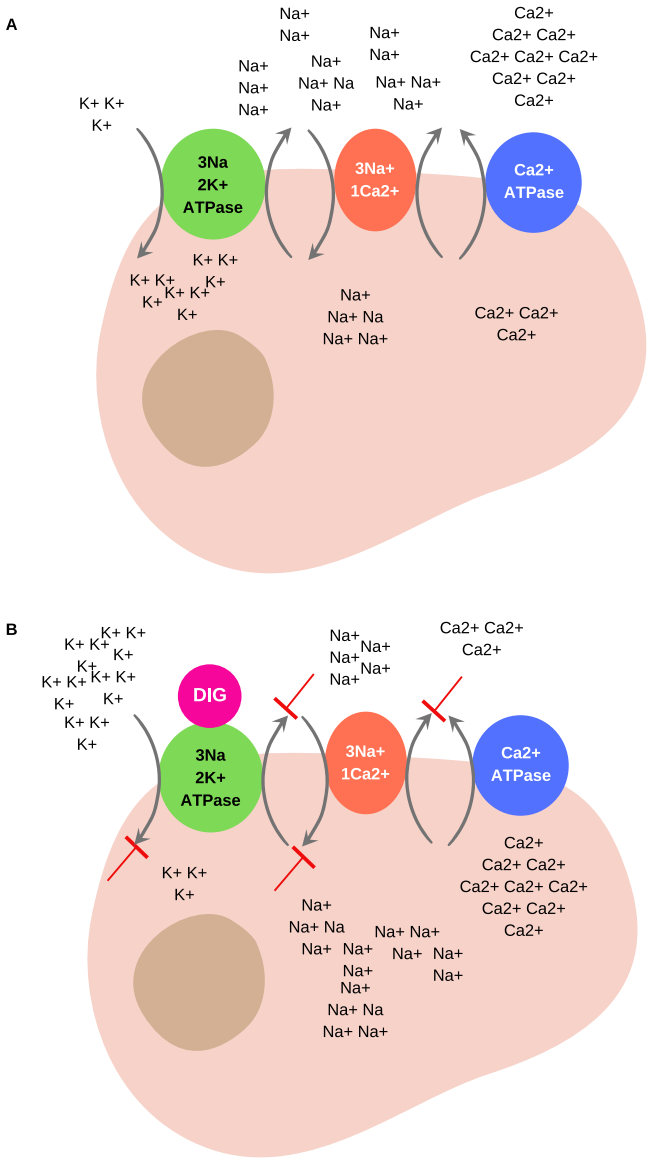

Fig. 2. Figure 2 Digoxin inhibits 3Na-2 K-ATPase. 3Na-2 K-ATPase = sodium-potassium-adenosine triphosphate pump, 3Na-2Ca2 + exchanger = sodium calcium antiporter, Ca2 + ATPase = calcium ATPase pump, DIG= Digoxin. (A) 3Na-2 K-ATPase is an ATP-dependent ion pump required for extracellular exchange of 3 Na+ ions for 2 K+ ions intracellularly. In normal physiological conditions, high extracellular Na+ and Ca^{2+} concentrations are maintained by the 3Na-2 K-ATPase and Ca-ATPase respectively. This facilitates passive movement of Na+ down its concentration gradient into the cell, which is required for export of Ca^{2+} via the Na-Ca^{2+} antiporter. (B) Digoxin inhibits activity of 3Na-2 K-ATPase, which increases intracellular sodium and calcium. Increased intracellular calcium mediates haemodynamic effects of reduced heart rate and increased heart contractility.

Recently, two studies demonstrated that digoxin has broad spectrum senolytic properties in mice (Guerrero et al., 2019; Triana-Martinez et al., 2019). Guerrero et al. (2019) demonstrated treatment of murine pituitary craniopharyngioma cells with digoxin reduced mRNA expression of CDKN1a (p21), and SASP cytokines IL-1b and IL-6. Triana-Martinez et al. (2019) studied the effect of digoxin treatment in mouse models of pulmonary fibrosis, showing 2 mg kg^{-1} concentration for 10 days reduced expression of CDKN1a and CDKN2a (p16). Evidence of digoxin altering pro-inflammatory cytokine profile in mouse models of age-related disease - including atherosclerosis (Shi et al., 2016), autoimmune arthritis (Lee et al., 2015) and Alzheimer's disease (Erdogan et al., 2022) - suggest digoxin could have senomorphic properties but further research is required to confirm this. We now outline the proposed senotherapeutic mechanisms of digoxin described in preliminary cellular and animal studies: upregulation of intrinsic apoptotic pathways, intracellular acidification and alteration of the T cell pool to ameliorate pro-inflammatory cytokine profile.

4.1. *Upregulation of intrinsic apoptotic pathways*

Apoptosis is regulated by extrinsic and intrinsic pathways. These pathways have been discussed elsewhere (Galluzzi et al., 2018; Kale et al., 2018; Krammer, 2000; Lawen, 2003; Singh et al., 2019), but in brief extrinsic pathways are mediated via extracellular TNF-receptors ("death receptors") whereas intrinsic pathways rely on intracellular mitochondrial signalling (Kale et al., 2018; Krammer, 2000; Singh et al., 2019). Digoxin promotes the activity of the intrinsic apoptotic pathways by promoting mitochondrial BCL-2 proteins which induce protease-mediated cell destruction (Guerrero et al., 2019; Kale et al., 2018; Singh et al., 2019) Fig. 3.

How digoxin promotes pro-apoptotic proteins is not fully understood. However, it is known that in addition to regulating ion concentration gradients, Na-K-ATPase functions as a signal transducer (Guerrero et al., 2019; Schoner and Scheiner-Bobis, 2007). Aberrant Na-K-ATPase signal transduction is implicated in cancer development by promoting phosphorylation of oncogenic proteins (EGFR, Src, MAPK) (Haas et al., 2002; Kometiani et al., 2005; Lin et al., 2015; Song et al., 2020). Digoxin-mediated inhibition of Na-K-ATPase signal transduction ameliorates tumour invasion and metastasis in several human cancer cell lines including breast (Kometiani et al., 2005), lung (Lin et al., 2015), and liver (Song et al., 2020). It is proposed that Na-K-ATPase located at the endoplasmic reticulum might act as a sensor of cell survival and apoptosis (Lauf et al., 2015, 2013). Anti-apoptotic proteins can bind with Na-K-ATPase (Lauf et al., 2015, 2013) so it is plausible that digoxin disrupts this binding by interrupting Na-K-ATPase signal transduction.

4.2. *Intracellular acidification*

Senescent cells by nature are more acidic (lysosomal SA-β-Gal is detected at pH 6 (Dimri et al., 1995)) and, compared to non-senescent cells, can withstand a lower intracellular pH before organelle dysfunction and stimulation of apoptosis (Triana-Martinez et al., 2019). Digoxin-mediated Na-K-ATPase inhibition not only reduces Na^{+}/Ca^{2+} exchange but effects the activity of Na^{+}-H^{+} antiporter (Lazdunski et al., 1985) Fig. 3. This results in accumulation of H+ and intracellular acidification (Triana-Martinez et al., 2019). By potentiating an unfavourable intracellular pH, digoxin provides the apoptotic "push" required for cell destruction (Triana-Martinez et al., 2019) Fig. 3 which senescent cells are more vulnerable to due to their acidic cytoplasmic environment.

4.3. *Amelioration of pro-inflammatory cytokine profile*

T cells are part of the adaptive immune system. Within the T cell

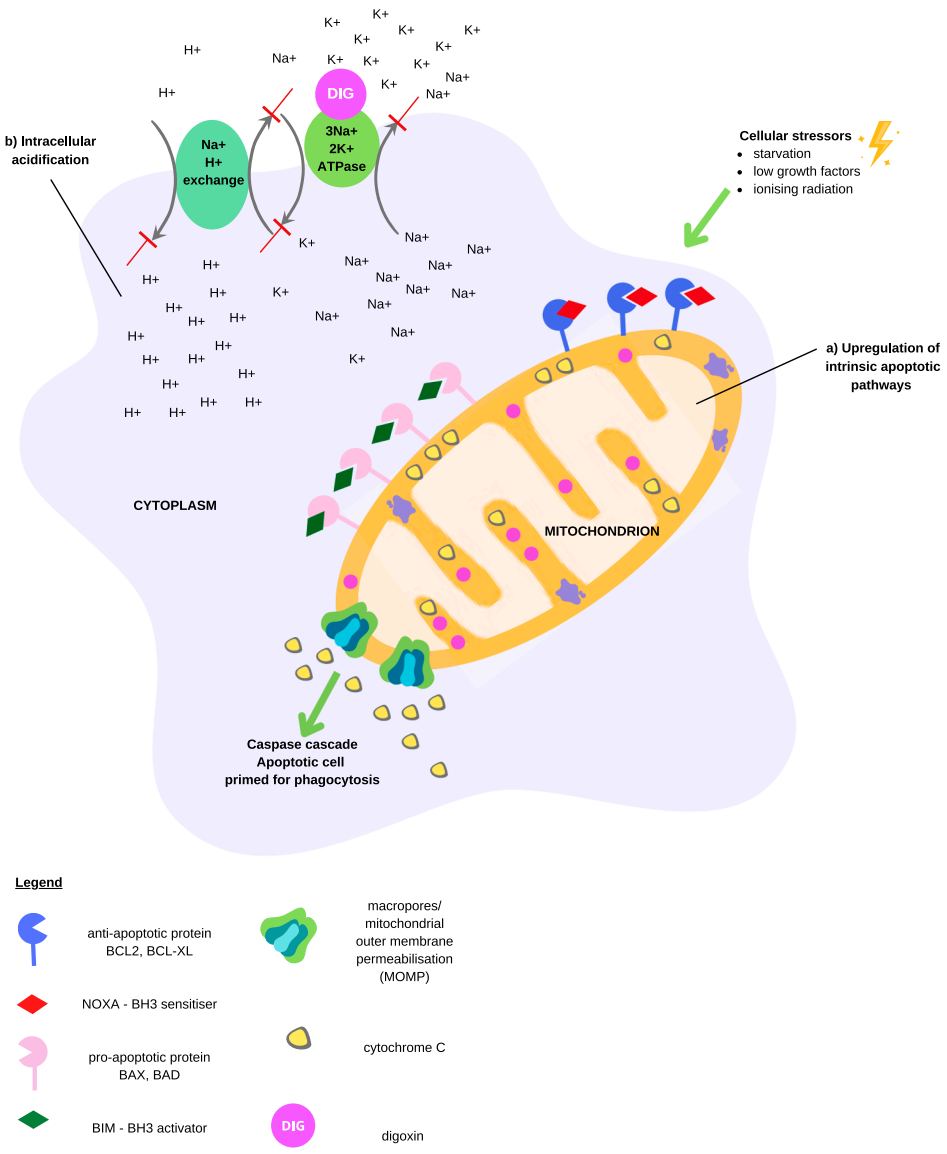

Fig. 3. Figure 3: a) Digoxin upregulates intrinsic apoptotic pathways. Cellular stressors such as starvation, altered growth factors and ionising radiation activate B Cell Lymphoma-2 homology domain 3 only (BH3) proteins, which act as activators or sensitisers (Kale et al., 2018; Singh et al., 2019). BH3 activators (e.g. BID and BIM) bind to mitochondrial pro-apoptotic proteins (e.g. BAX and BAK), which "hole punch" the outer mitochondrial membrane via macro-pores (Kale et al., 2018; Singh et al., 2019). The leaky mitochondrial membrane (Mitochondrial Outer Membrane Permeabilisation, MOMP) enables release of caspase-activating proteins, such as cytochrome C, and subsequent caspase mediated cell destruction (Kale et al., 2018; Singh et al., 2019). BH3 sensitisers (e.g. BAD and NOXA) inactivate mitochondrial anti-apoptotic proteins (e.g. BCL-2, BCL-X_L) on binding (Kale et al., 2018). b) Digoxin promotes intracellular acidification. Digoxin inhibits Na-K-ATPase which prevents extracellular pumping of Na+ , reducing the concentration gradient driving Na+ into the cell. This subsequently lessens the activity of the Na-H exchanger, which also utilises passive movement of Na+ down its concentration gradient to export H+ extracellularly, resulting in increased intracellular H+ and acidification of the cell (Lazdunski et al., 1985; Triana-Martinez et al., 2019). Senescent cells by nature have a lower pH, an unfavourable biochemical environment for organelle function, which is potentiated by digoxin (Triana-Martinez et al., 2019). Digoxin provides an apoptotic "push" to pathological cell death.

family are different subtypes, each having unique functions. Th17 cells, T helper cells, are a subset of pro-inflammatory CD4 + T cells which produce cytokines IL-6, IL-17A, and TNF α (Carrasco et al., 2022; Lee et al., 2015; Shi et al., 2016; Shirakawa et al., 2016) Fig. 4. In contrast, T reg cells suppress cytokine production and prevent overactive immune responses driving autoimmune disease (Carrasco et al., 2022; Vignali et al., 2008). However, IL-6 can induce conversion of T reg cells into a subtype of CD 4 + T helper cells, Th17 cells (Shi et al., 2016; Vignali et al., 2008) Fig. 4.

Mouse studies demonstrate digoxin can ameliorate chronic low grade inflammatory processes which contributes to ageing, "inflammageing" (Ferrucci and Fabbri, 2018), by altering the pool of T cell populations (Erdogan et al., 2022; Huh et al., 2011; Lee et al., 2015; Shi et al., 2016). Digoxin increases T reg cell frequencies and reduces the conversion of T reg to Th17 cells (Shi et al., 2016; Wu et al., 2013) Fig. 4. The proposed mechanism is antagonism of RORγt, a transcription factor that drives metabolic syndrome and inflammatory diseases (Huh et al., 2011; Lee et al., 2015; Shi et al., 2016; Wu et al., 2013) Fig. 4.

In apolipoprotein E-deficient mice fed a fatty diet, a model for atherosclerosis, digoxin treatment for 12 weeks reduced atherosclerotic plaque formation, plasma lipid levels and altered cytokine profile by reducing pro-inflammatory IL-6, IL-17A, TNF-α and increasing anti-inflammatory IL-10 levels (Shi et al., 2016). Similar alteration in cytokine profile with digoxin treatment was demonstrated in mouse models of autoimmune disease (Huh et al., 2011; Lee et al., 2015). Digoxin treatment for three weeks duration in mouse models of Alzheimer's disease reduced brain TNF-α levels after a (Erdogan et al., 2022). These studies did not delineate whether pro-inflammatory cytokines were produced by senescent or non-senescent T-cells. However senescent T cells are implicated in the pathogenesis of autoimmune disease, atherosclerosis and dementia (Jung and Shin, 2017; Lu et al., 2022; Saez-Atienzar and Masliah, 2020; Yu et al., 2016). Therefore, it is likely that observed alteration of cytokine profile in these studies is, at least in part, accounted by senomorphic properties of digoxin - further research in animal models is required to confirm this mechanism.

5. Discussion: future prospects and challenges for repurposing digoxin for geroprotection

Repurposing of commonly used drugs with secondary geroprotector properties is a proposed strategy that could promote regulatory approval and incorporation of geroprotector drugs into clinical practice (Kulkarni et al., 2022). Clinician familiarity supports repurposing digoxin for geroprotection - digoxin is widely prescribed in older adults to treat AF

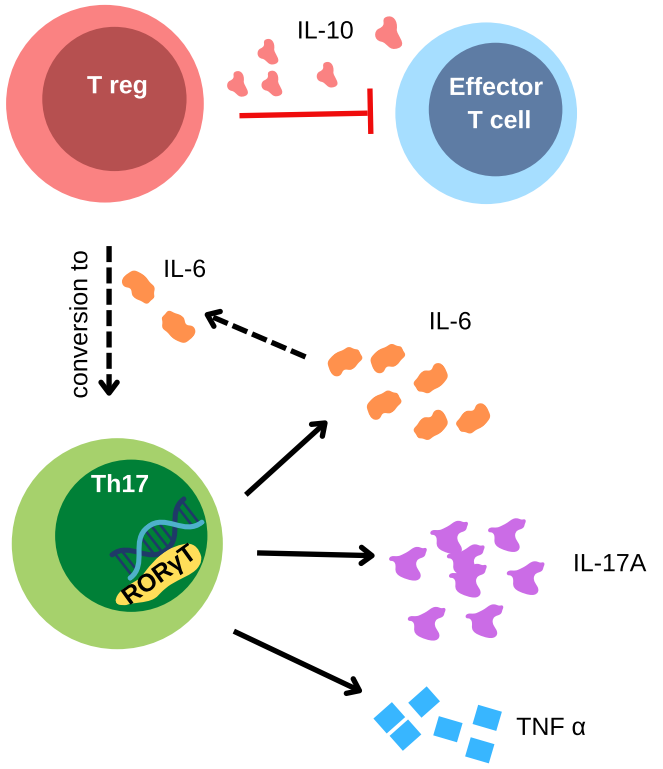

Fig. 4. FIG4.PDF: Digoxin antagonises activity of RORγt. Th17 cells are characterised by release of pro-inflammatory cytokines including IL-6, IL-17A and TNF α. T reg cells ensure peripheral tolerance via IL-10-mediated suppression of effector T cells, preventing over-active immune processes driving autoimmune disease. IL-6 mediates conversion of T reg cells to Th17 cells. Digoxin increases T reg cells and prevents their conversion to Th17 cell by RORγt antagonism.

and heart failure, with over 2.8 million prescriptions per year in primary care in England (Oxford, 2022). In addition digoxin is safe within a narrow therapeutic range (Adams et al., 2005; Rathore et al., 2003) however a limiting factor in older adults, particularly those with renal disease, is increased susceptibility to toxicity which can occur at therapeutic serum digoxin concentration (Ziff and Kotecha, 2016).

Historically, digoxin has been prescribed in sicker patients with frailty and multimorbidity, resulting in prescription bias in observational studies and the incorrect perception that digoxin use is associated with excess mortality risk (Ziff and Kotecha, 2016; Ziff et al., 2015). In contrast, analysis of all randomised controlled trial data shows that digoxin has a neutral effect on mortality (Ziff et al., 2015). In the real-world, rate-control decisions are often based on clinician's previous clinical experience and personal judgement due to lack of robust evidence (Kotecha et al., 2020, 2017).

The prospect that digoxin could be repurposed as a senotherapeutic is exciting. However further cellular and animal studies are needed to identify whether digoxin works on ageing mechanisms at low dose before age-related disease develops. Drug regulatory bodies have not yet established processes for validating benefit of proposed geroprotector agents in humans (Kulkarni et al., 2022). Ultimately, high quality randomised controlled trials involving older adults are required before geroprotectors could be incorporated in clinical practice. These trials are often costly and time-intensive, but given digoxin has been in clinical use for a long time this offers potential for simulated trials within health record data (Wedlund and Kvedar, 2021) – particularly prior to 2000 when digoxin was more commonly used in "younger older" people before multimorbidity, thereby providing a long follow-up period.

Geroscience and geroprotector drug research is a rapidly evolving field, with potential to create a paradigm shift in practice of clinical medicine - from mitigation of disease and risk factors to targeting the cellular processes that drive unhealthy ageing. However, what geroscience researchers may determine as "successes" may not be shared by older adults - we do not know the acceptability of geroprotector drugs to older adults, nor what outcome measures are most important to them. Researchers must integrate patient and public involvement throughout the whole research process to facilitate progress whilst remaining "gero-" centred.

Competing interests

Dr Helena Lee is a recipient of a NIHR Academic Clinical Fellowship and British Geriatric Society Specialty Registrar Research Start Up Grant. Dr Daisy Wilson is a recipient of a NIHR Clinical Lectureship. Dr Bunting was the research fellow for the RATE-AF trial funded by the NIHR (NIHR CDF-2015-08-074) and has been awarded a grant from the University of Birmingham's British Heart Foundation Accelerator Award (BHF AA/18/2/34218) and the British Heart Foundation Fellowship Scheme (FS/CDRF/21/21032). Prof. Kotecha reports grants from National Institute for Health Research (NIHR CDF-2015-08-074 RATE-AF; NIHR130280 DaRe2THINK; NIHR132974 D2T-NeuroVascular; NIHR203326 BRC), the British Heart Foundation (PG/17/55/33087, AA/18/2/34218 and FS/CDRF/21/21032), the EU/EFPIA Innovative Medicines Initiative (BigData@Heart 116074), EU Horizon (HYPERMARKER 101095480), and the European Society of Cardiology supported by educational grants from Boehringer Ingelheim/BMS-Pfizer Alliance/Bayer/Daiichi Sankyo/Boston Scientific, the NIHR/University of Oxford Biomedical Research Centre and British Heart Foundation/University of Birmingham Accelerator Award (STEEER-AF NCT04396418). In addition, he has received a research grant to his institution from Pfizer; and research grants and advisory board fees from Bayer, Amomed and Protherics Medicines Development; all outside the submitted work. Dr Thomas Jackson is a recipient of a NIHR research grant investigating multimorbidity clusters. The views expressed are those of the author(s) and not necessarily those of NIHR.

Data Availability

Data will be made available on request.

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