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New Usages for Known Drug Molecules

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Abstract

As the development of drugs becoming more and more expensive and complicated, it is appeared to be rather easy to find a new effect for present active molecules. There are some good examples such as aspirin. It has been re-proposed for cardiovascular system as an anticoagulant and a protectant against to arteriosclerosis. Sildenafil is an interesting example because it is phosphodiesterase 5 inhibitor and it enhances blood vessel diameters. It has been re-proposed for pulmoner hypertension later and it has been found to be very effective and useful. Another good example is raloxifene. Although it has been initially proposed for the treatment of osteoporosis, it has been found to be effective on breast cancer cells. Therefore pros and cons of drug re-proposing and all these will be discussed with some examples from the literature. Key elements of drug re-proposing will be given and results will be discussed in this article.

Key words: Drug repurposing, re-proposing, new indication, new usage

Introduction

According to the search of literature bases; there are 23 papers for 2018, 519 papers for 2017, 365 papers for 2016, 301 papers for 2015 and 199 papers for 2014 available related to the topic "Drug repurposing" so far. The attention to the topic and drug repurposing is increasing in popularity. In fact the term "Drug repurposing" refers to application of therapeutic to a new disease indication, holds promise of rapid clinical impact on a lower cost. Repurposing generally tries to find a new usage of drugs or molecules that are already approved to treat a certain disease or condition because they are already proven to be safe. Discovery of a new molecule for a disease is becoming more and more expensive, complicated and time consuming efforts because guidelines require more evidence and procedures, paper works is increasing for licensing and scientific barriers for proving its safety and efficacy. These development efforts and spending times are called "bottlenecks" in the therapeutic development process (1).

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Delays and all these barriers mean that translation of a promising molecule into an approved drug often takes more than 10-15 years. It is crucial to advance strategies to reduce all these barriers, decrease costs and improve success rates. Drug repurposing appears to be one such strategy (2). Many chemical agents have been approved of other uses already and have been tested for humans for years, so detailed information is available on their pharmacology and potential toxicity. Drug repurposing to build upon previous research and it needs less development efforts; a new candidate molecule for a new therapy could be ready for clinical trial, therefore over all process will be shorter. At the end, if it is approved, their integration into health care will be very fast (3,4). Drug repurposing is also increasing because of increasing knowledge about molecular biology advances and physiopathological mechanisms of diseases. When an important enzymes or physiopathologic mechanism for a specific disease is found, a known enzyme inhibitor or related compound can be used to control this disease. In fact drug repurposing is a relatively large subject and there are number of papers related to drug repositioning or repurposing and identifying, developing new uses for existing drugs in the literature (5) especially from the pharmaceutical chemistry or molecular, structural point of view but this review is aiming to focus on sildenafil from general perspective. Although there are number of applications, well known issues and classical examples or published clinical trials related to sildenafil uses for hand-foot skin reaction, solid tumors, cirrhosis, rheumatoid arthritis, diabetes and so on; this review is focusing on rather interesting and selected applications, experiences and the results.

Some examples for proposing new indications for drug molecules

Aspirin

Aspirin, acetyl salicylic acid has been manufactured and marketed since 1899 as nonsteroidal anti-inflammatory drug (6). Atherothrombosis, fatal myocardial infarction, ischemic stroke and similar diseases were causing many deaths these days and a key mechanism behind that to prevent all these health problems was appeared to be the use of pharmacological agents to counteract the process of clot formation. Almost 60 years later, the antithrombotic potential of aspirin as an antiplatelet agent was noticed and aspirin was started to use for the primary prevention to avert the onset of cardiovascular disease by targeting its natural causes and risk factors (6). At a different level, secondary prevention strategy was also announced which includes strategies and therapies that address preclinical or clinical evidence of cardiovascular disease progression. Aspirin can reduce the mortality and risk of recurrent vascular events in the secondary prevention of ischemic stroke (7). Recent reports and current guidelines recommend loading of aspirin between 160 and 325 mg for patients having acute ischemic stroke within 48 hours (8,9). Although the side effects of aspirin (e.g., gastrointestinal upset or bleeding events) are known to be dose dependent, a wide range of aspirin doses to

seem equally effective fir exerting it's antithrombotic effect (10,11).

Some other effect of aspirin has been also reported such as the prevention and treatment of fetal growth suppresses the production of prostaglandins and thromboxanes through its irreversible inactivation of the cyclooxygenase enzyme. Thromboxane is a powerful vasoconstrictor and prothrombotic antiplatelet agent. Low-dose, long-term aspirin use irreversibly blocks the formation of thromboxane A2 in platelets and platelet aggregation inhibited. More recently, novel cytoprotective and antioxidant mechanisms of aspirin have been observed that are independent of cyclooxygenase inhibition (12). The release of soluble endoglin (13,14) into the maternal circulation reported to cause some endothelial dysfunctions. A feature of the placenta-mediated complications in pregnancy or in particular in preeclampsia and an imbalance in vasoactive factors such as endothelin (15), nitric oxide (16) and prostacyclin (17), results in reduced vasodilatation or increased vasoconstriction. Aspirin acetylates endothelial nitric oxide synthase, leading to nitric oxide release from the vascular endothelium (18). In addition, aspirin increases the activity of oxygens in endothelial cells and catabolization occurs, all these leads to a reduction in oxidative stress, injury, and inflammation (19). Aspirin has a number of effects on vascular level that may prevent fetal growth restriction. For many years it was understood that aspirin affect the blood system cells and related functions. Repurposing ideas of other drugs for fetal growth restrictions have been also reported. Because, the development of new drugs or the testing of unused drugs for the treatment of fetal growth restriction pregnancy is again difficult and costly, the repurposing of existing drugs that have a known safety profile in especially pregnancy is important and an exciting area. Proton pump inhibitors such as esomeprazole have already long-term safety data about the treatment of gastric reflux in pregnancy. *In vitro* studies showed that proton pump inhibitors decrease soluble endoglin and improve markers for endothelial dysfunctions (20). Similarly melatonin, an endogenous lipid-soluble hormone produced by the pineal gland, exerts its powerful antioxidant effect directly by scavenging reactive oxygen species. Melatonin acts indirectly by increasing the expression of antioxidant enzymes such as glutathione peroxidase and glutathione reductase. Melatonin can cross the placenta (21) and the fetal blood brain barrier (22) and it can potentially protect the developing fetal brain and heart from the damage by oxidative stresses.

Minoxidil

Minoxidil was originally developed for treating hypertension but its stimulation effect on hair growth in androgenetic alopecia was discovered afterwards (23). Minoxidil was found to be effective to increase blood circulations around hair follicles and it enhances the conversion of testosterone to other type of androgen molecules (24). Topical application of minoxidil solution enhances the anagen phase of hair follicle (25). It has been suggested that the dermal papilla cells were likely the target cells for minoxidil (26). It

has been also shown that minoxidil maintains vascularization in dermal cells *via* stimulation of VEGF expression in androgenic alopecia (27). Although the exact role of minoxidil on anagen phases and how it induces signaling pathway is still unknown, it has been using successfully to overcome androgenic hair loss problem. The vasodilator effect of minoxidil accepted to be because of its sulfate metabolite. It has been shown that minoxidil sulfate affects muscular smooth muscles and it rapidly dilates the vascular smooth muscles and blood supply increases for hair follicle (28-31). Sulfation mainly catalyzed by sulfotranferase and minoxidil indirectly enhances the blood supply for hair follicle and induces the hair growth (32-4).

Raloxifene

Raloxifene was initially developed for the prevention and treatment of osteoporosis in women (35). Osteoporosis is 'a disease characterized by low bone mass and deterioration of bone architecture leading to reduced bone strength and increased the risk of bone fracture. Osteoporosis is implicated in most unexpected fractures occurring in the elderly population. It is estimated that approximately 40–47% of all women at the age of 45–50 years will suffer at least one osteoporotic fracture during the remainder of their lifetime (35). Such fractures often have considerable consequences for the patient owing to increased morbidity and pain, loss of independence, reduced life expectancy, rehabilitation and nursing home care etc. Raloxifene is one of the selective estrogen receptor modifiers (SERM). SERMs bind to the estrogen receptor, but not at the ligand pouch, and it can change the receptor conformations. These alterations, the affinity with a number of tissue-specific transcription factors (co-activators and co-repressors), leading to estrogen agonistic effects for some tissues, such as bone, and antagonistic effects in other tissues, for example breast. This group of compounds namely tamoxifen, raloxifene and several other drugs subjected to many studies. Only raloxifene approved for the prevention and treatment of osteoporosis among them[35]. Since then raloxifene has been discovered, it has been tested in many studies for repurposing; for instance it has been tested for fibromyalgia in menopausal women (36), on bile compositions or depression and cognition in ovariectomized animals (37,38), aortic atherosclerotic lesions of female rabbits submitted to ovariectomy and hypercholesterol diet (39), chemoprevention of breast cancer in high risk women (40), modulation of estrogen receptor to inhibit proliferation and migration of prostate cancer cells (41).

Recently, raloxifene has been selected as a model drug and a series of raloxifene-loaded liposome and cochleate formulations were prepared in literature (42). *In vitro* release studies and *in vivo* tests have been studied and breast cancer cell lines (MCF-7) have been used to find the most effective formulation. The highest antitumor activity was reported to be observed with raloxifene-loaded cochleates containing DM-β-CD and MMP-2 enzyme which is responsible for breast cancer has also found to be inhibited.

Sildenafil

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Erectile dysfunction (ED) is defined as "the inability to achieve and/or maintain a sufficient penis erection for satisfactory sexual performance and has been associated with both organic and psychogenic causes (43). Arterial damages, problems of smooth muscles and fibrous tissues result in altered blood flow and all these thought to be the most common cause of erectile problems (44). Some psychogenic causes of ED include depression, anxiety, relationship problems, and schizophrenia (45,46). The worldwide prevalence of sexual dysfunctions has been estimated to be approximately 322 million cases by 2025 (44). In the general, 10% to 20% of men are expected to experience erectile problems of normal population, with an overall incidence of 30% and 18.4% in the European Union and the United States, respectively (47,48). In these populations, the incidence of erectile problems was reported to be the highest in the elderly people (> 70 years) (64% and 70.2% in Europe and US) followed by the age group of 60 to 69 years (38% and 43.8%, respectively) and 40 to 59 years (25% and 14.8%, respectively) (47,48). ED and problems have a significant impact on the quality of life of patients. Men with this problem have been reported to have lower levels of physical activity, emotional satisfaction, and general happiness and have also been documented to exhibit role limitations because of the impact it has on their emotional well-being (49).

Several therapeutic options are available for the treatment of ED. One of these options is based on the use of PDE type5 (PDE5) inhibitors and a good example drug is Sildenafil. Sildenafil citrate selectively inhibits PDE5. It is the first oral medication used for the treatment of ED (50). Sildenafil citrate is a sparingly soluble water soluble compound but it has sufficient membrane permeability. The drug molecule can be absorbed quickly and it comes under class 2 of the biopharmaceutical classification system (BCS). The oral bioavailability of Sildenafil citrate is reported to be quite low. It is quickly absorbed from the intestine but it provides only 40% absolute bioavailability. It has a late onset of action. Sildenafil citrate, marketed as Viagra®, and approved by the Food and Drug Administration (FDA) for the treatment of ED (51-53). Once it was approved and it has an effect on PDE5 it is possible to have some other effects, therefore some new investigations were started because PDE5 is a cyclic nucleotide PDE which is present in many tissues such as neurons, smooth muscle cells, and the vascular walls of the brain. It inactivates the second messenger cGMP by hydrolysis to GMP (54-58). Sildenafil inhibits PDE5 and therefore the half-life of endogenous cGMP is prolonged (58). Sildenafil has the ability to relax vascular smooth muscle cells, leading to dilation of the blood vessels thus increasing downstream blood flow can be obtained (59,60).

Successful attempts for sildenafil

Treatment of pulmonary hypertension

Pulmonary hypertension is a type of high blood pressure that affects arteries in the lung and right side of the heart. In one form of pulmonary hypertension, tiny arteries in the lung, called pulmonary

arterioles, and capillaries become narrowed, blocked or destroyed. This makes harder for blood to flow through the lung, and raises pressure in arteries of the lung (61). As the pressure builds up, lower right chamber of the heart (right ventricle) must work harder, eventually causing your heart muscle to weaken and fail. Some forms of pulmonary hypertension are serious conditions that become progressively worse and are many times fatal. Although some form of pulmonary hypertensions aren't curable, treatment with some drugs can help to improve some symptoms and increases the quality of life (61). According to the latest reports of Harvard Men's Health Watch journal at the August 2007 issue, sildenafil is now marketed with a trade name for specifically treatment of pulmonary hypertension (62). This drug was developed to use for the treatment of pulmonary arterial hypertension in adults to improve exercise ability and to postpone clinical worsening. This delay in clinical worsening was reported to be demonstrated when this drug was used (63). Studies were reported to be established the effectiveness for short-term (12 to 16 weeks), and included predominately patients with New York Heart Association (NYHA) Functional Class II-III symptoms and idiopathic etiology (71%) or associated with connective tissue disease (25%) (63). FDA recently approved this drug for the treatment of pulmonary hypertension but there is concern raised especially used this drug in children. FDA Drug Safety Communication clarified warning about pediatric use of this drug (sildenafil) for pulmonary arterial hypertension after that. In early 2014, FDA was clarified their previous recommendations related to prescribing this drug (sildenafil) for children with pulmonary arterial hypertension (PAH) because this drug has a FDA-approval for only to treat PAH in adults, not in children (64). However, health care professionals are free to consider whether the benefits of treatment with the drug are likely to outweigh its potential risks of each patient. In fact FDA revised this drug label in August 2012, adding a warning and stating that “use of this drug, particularly chronic use, is not recommended in children”. This recommendation was reported to be made based on an observation of increasing mortality with increasing the doses of this drug in a long-term clinical trial in pediatric patients with PAH (65). The purpose of the recommendation was to raise the awareness of clinical trial results showing a higher risk of mortality in pediatric patients taking a high dose of drug when compared to pediatric patients taking a low dose (66). This recommendation was not intended to suggest that this drug should never be used in children; however, health care professionals should consider the risk and benefits, this was just a warning.

Treatment of ischemic stroke and neuroprotective effects

Results of some preclinical studies suggest that sildenafil as a PDE5 inhibitor may improve functional outcome following ischemic stroke. A detailed and systematic review has been done aimed to evaluate the effects of selective PDE5 inhibitors in animal models of brain ischaemia (64). A systematic search of Medline, Embase, and

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The Cochrane Library was reported to be performed including studies in English assessing the effects of selective PDE5 inhibitors. 32 publications were included describing outcome in 3646 animals. Neuroprotective effects of PDE5 inhibitors were found to be dependent on the NO-cGMP-PKG-pathway. These included reduced neuronal apoptosis, oxidative stress, and neuroinflammation (65). Interestingly and controversially sildenafil did not find to be effective against 6-hydroxydopamine lesioned rats in another study. Sildenafil did not find to protect against 1-methyl-4-phenyl-1,2,3,6-tetrahidropyridine (MPTP)-induced dopamine depletion in the striatum (66). Sildenafil may be more effective and can be a neuroprotective especially after the stroke because PDE5 inhibitors can increase angiogenesis and elevate regional cerebral blood flow in the ischemic penumbra, and improved functional recovery. Some reports indicated that PDE5 inhibitor treatment can reduce the lesion volume, others had no effect. Treatment was found to be effective when administered within 24 h post-ischemias, though treatment delayed to seven days improved outcome in one study (65). The effect of sildenafil may be defined as neurorestorative rather than neuroprotective. There is currently limited evidence for the effects of PDE5 inhibitors in humans; more clinical trials and evidences still needed.

Synaptogenetic effect and synapse protection

Sildenafil showed protective effects on synapse damages, including post stroke axonal swelling and demyelination (67). After having an ischemic stroke, an increase in coupling of postsynaptic density protein changes was reported to be seen. This protein is a post synaptic membrane bound protein which functions as a scaffold for clustering of receptors, ion channels, and signaling proteins thus it is reported to be important for synapticplasticity (68). Reduced coupling of this protein and changes in post-ischemia can therefore be interpreted as a reduced synapse damages. Sildenafil administered to rats were found to have a reduced loss of synaptophys in (this is a marker for number of synapses) and decreased coupling of postsynaptic density protein changes were seen, those effects seen together with preserved synapse structure (67,68). Sildenafil found to be significantly increased brain concentration of many factors such as growth factors tropomyosin-related kinase B, brain-derived neurotrophic factor, tropomyosin-related kinase A, and nerve growth factor involved in synapse functioning in rats (67).

Treatment of Alzheimer's disease (AD)

Sildenafil and analogues also improved performance of the memory measured with Morris water maze and aversive radial maze post-stroke (69). The positive effects of them on functional outcomes were observed. Recent research results in molecular biology and technology, multiple credible hypotheses about the progress of AD have been proposed; multi-target drugs have emerged as an innovative therapeutic approach for AD. Current clinical therapy for AD patients is mainly palliative treatment targeting acetylcholinesterase. Inhibition of PDE-5A

has recently been validated as a potentially novel therapeutic approach for AD. In a work in the literature, series of new compounds have been designed, synthesized and evaluated as dual cholinesterase and PDE inhibitor. These compounds have been designed and biological results revealed that some of these compounds display good biological activities against acetylcholine esterase and PDE-5A (70).

High levels of amyloid-beta peptide (A beta) have been found to be related to AD and its pathogenesis. However, in the healthy brain, low physiologically relevant concentrations of A beta are necessary for long-term potentiation and memory. As GMP plays a key role in these processes, the cyclic nucleotide cGMP has an influence on A beta levels and function during long term potentiation and memory. It has been demonstrated in the literature that the increase of cGMP levels by the PDE-5 inhibitors like sildenafil induces a parallel release of A beta due to a change in the approximation of amyloid precursor protein (APP) and the beta-site APP cleaving enzyme (71). Their data suggest that cGMP positively regulates A beta levels in the healthy brain which, in turn, boosts synaptic plasticity and memory.

An improvement on vascular and metabolic function of patients with AD has been demonstrated in the literature[72]. Patients with AD have alterations in cerebral hemodynamic function including reduced cerebral blood flow and cerebral metabolic rate. Therefore, improved cerebrovascular function reported to be an attractive goal for pharmaceutical intervention in AD. Complete cerebral blood flow, cerebral metabolic rate of oxygen and cerebrovascular reactivity data were obtained from patients. Cerebral blood flow and cerebral metabolic rate of oxygen have been found to be significantly increased following sildenafil administration. Cerebral blood flow was most pronounced in the bilateral medial temporal lobes. Cerebrovascular rate significantly decreased after administration of sildenafil. Their data suggest that a single dose of sildenafil improves cerebral hemodynamic function and increases cerebral oxygen metabolism in patients with AD (72).

A model of aging in mice reported to be displayed many pathological indicators of AD. In this model, sildenafil administration was found to be attenuated learning and memory impairments (73). The increased expression of β-site amyloid precursor protein cleaving enzyme 1 was also found to be reduced by sildenafil, this effect was also found to be paralleled to decreases in the activities of this enzyme modulator. All findings highlight the therapeutic potential of sildenafil in AD pathogenesis (73).

Relaxing cerebral vasospasm

A hemorrhage from aneurysmal subarachnoid tissue is a significant health care problem with high morbidity and mortality. According to the latest reports up to 70% of patients could die or be permanently disabled because of cerebral vasospasm and some other problems (74). Although prognosis of this condition is dependent on a number of factors such as age, current neurological status, and developed cerebral ischemia (75). The mechanisms underlying this cerebral

ischemia is still not exactly known, the most accepted responsible factor is cerebrovascular vasospasm and related consequences (76). Unfortunately, up to date quite few therapies have proven to be effective for the prevention and treatment (77). The results of a Phase I safety and proof-of-concept trial in the literature indicates and assessing the use of intravenous sildenafil administration in patients with cerebrovascular spasm shows that sildenafil is safe and well tolerated in the setting of subarachnoid hemorrhages and following cerebrovascular spasms (78). Furthermore, the authors concluded that their angiographic data proved sildenafil has a positive impact on human cerebrovascular spasm.

Treating the dysfunctional placenta

It was reported that placental dysfunction underlies major obstetric diseases such as pre-eclampsia and fetal growth restriction (79). Whilst there has been a little progress in prophylaxis of this condition, there are still no successful treatments for placental dysfunction. However, there are well-described *in vitro* systems for studying the human placenta available and there are some animal models described for studying preeclampsia and fetal growth restriction. Sildenafil usage and its effect placental dysfunction have been studied. As it has been mentioned earlier, sildenafil is a phopshodiesterase-5 inhibitor which blocks the breakdown of cGMP and so enhances nitric oxide (NO)-mediated vasodilation. Sildenafil could be useful to improve perfusion through the utero-placental circulation. A systematic review of a small number of clinical and animal pregnancy studies in 2007 concluded that sildenafil showed no teratogenic or other adverse effects in animals or any women (80). Other investigators were than investigated whether sildenafil would improve vasodilation in small myometrial arteries. They found that vessels from fetal growth restricted placentas showed increased artery vasoconstrictions and decreased endothelial-dependent vasodilation as compared to normal placentas and these effects were reversed successfully by sildenafil (81). Interestingly another researcher reported that sildenafil on a sheep model of fetal growth restriction produced by single umbilical artery ligation had no effect on uterine blood flows, and in fact sildenafil increased the heart rate of fetus (82) but It was found to be clear about the pathophysiology of placental dysfunction and clinical observation that the disease has different phenotypes in different from subject to subject (83). Therefore this was found to be the reason for getting variable results.

Treatment of infertility

It has been reported that vaginally administered sildenafil could lead to an improvement in uterine blood flow and, in conjunction with controlled ovarian hyperstimulation, led to estrogen induced proliferation of the endometria lining in patients with in vitro fertilization failure associated with poor endometrial development. According to the literature, patients need to receive vaginal sildenafil administration at a dose of 25 mg 4 times a day for effective treatment (84,85).

Therefore, a controlled release vaginal sildenafil tablet formulation was proposed and developed to reduce administration discomfort and effective therapy was targeted (85). These developed formulations were found to be beneficial for both scientists and patients with infertility problems caused by a poor endometrial development.

Effects on fat tissue

It has been investigated that whether the short-term treatment of sildenafil can induce browning of subcutaneous white adipose tissue in human adults in literature (86). The results showed that sildenafil significantly decreased adipocyte size, increased the expressions of proteins and mRNA, mitochondrial density, and leak respiratory capacity in subcutaneous white adipose tissue ($p<0.05$). Sildenafil also increased plasma cGMP and catecholamine concentrations ($p<0.05$), and consequently activated the expressions of vasodilator-stimulated phosphoprotein but sildenafil did not found to activate typical brown fat (86). It was concluded that sildenafil may be a promising treatment for metabolic disease.

Treatment of Reynold phenomenon

Raynaud phenomenon is a condition which manifests as recurrent vasospasm of the fingers and toes and generally occurs in response to stress or cold exposure (87). The phenomenon is named first time by Maurice Raynaud, who was a medical student. This health condition was defined with a first case in 1862 as a vasospasm characterized by pallor, cyanosis, suffusion, and a sense of fullness or tautness, which may be painful (88). Secondary Raynaud phenomenon should be distinguished from primary Raynaud phenomenon (Raynaud disease). They are distinct disorders that share a similar name. Raynaud disease is characterized by the occurrence of the vasospasm alone, with no association with another illness. Secondary Raynaud phenomenon is a designation usually used in the context of vasospasm associated with another illness, most commonly an autoimmune disease (87,88). Topical vasodilators can act as an adjuvant therapy for Raynaud phenomenon. Several trials have shown the benefits of topical nitrates (89,90). Nifedipine cream versus sildenafil cream was tested for patients with secondary Raynaud phenomenon in a randomized, double-blind, controlled pilot study (91). The vasodilator efficacies of topical 10% nifedipine versus 5% sildenafil in subjects with secondary Raynaud phenomenon associated with connective tissue disease were compared. It was found that 5% sildenafil cream improved the digital arterial blood flow in patients with secondary Raynaud phenomenon, suggesting local vasodilatation. Results of the study showed that topical sildenafil can significantly improve arterial blood flow in patient's skin with secondary Raynaud phenomenon (91).

Wound healing

PDE-5 is an enzyme that inactivates cGMP and regulates the balance of nitric oxide. Nitric oxide is an important molecule synthesized during wound repair. In some *in vivo* studies it was evaluated the effect of sildenafil, known to have a role in regulating the effect of nitric oxide in perfusion, on the

wound healing process under ischemic conditions. Sildenafil has been tested with animal studies and some histopathological analysis showed sildenafil significantly reduced re-epithelialization, neovascularization, amount of granulation tissue, and number of inflammatory cells (92) but controversially it has been recently reported that sildenafil increases nitric oxide release and accelerates wound healing and tissue perfusion; increases granulation in the literature (93). Another study dealing with the possible benefits of combining biodegradable polymers with sildenafil in wound healing in rats and their results showed that in spray dried formulation of sildenafil containing chitosan powder and its gel form could be promising wound healing promoters (94). Another study also supports their results and sildenafil containing gel topical applications decreased the defect areas in a dose independent manner (95). Sildenafil application was also increased vascularity and strength of the wound. Sildenafil was also administered orally to rats and the treatment with sildenafil showed that full thickness defects and chronic wounds with low incidence of side effects and morbidity were observed (96). Authors reported that oral sildenafil administration is a cost- effective and full thickness defects and chronic wounds can be treated with low incidence of side effects and morbidity.

Unsuccessful attempts for sildenafil

Anti-fatigue effect

Fatigue effect is one of the many effects attributed to fatigue, where the individual gradually becomes less and less efficient, even when completing minor tasks that they have completed prior giving rise to boredom. "Fatigue" is a condition which develops and progresses over time, the fatigue effect will lead to a loss in productivity and general tiredness. Anti-fatigue effect is an effect which reverses this kind of tiredness and loss in productivity. Fatigue is one of the most frequent and most disabling non-motor problems and results in the negative impact on cognitive and physical function, and quality of life in patients with the Parkinson's disease (66). Morning tiredness increases the risk of erectile dysfunction due to fatigue and a decrease in libido. Chronic fatigue reported to be occurred with aging, depression, diabetes, and Parkinson's disease and is one of the most common symptoms of primary care (97-99). There are very few pharmacological drugs or therapies available in the treatment of fatigue but sildenafil re-proposed for the treatment of fatigue (66). Sildenafil exerts its action through PDE-5 inhibition and it has shown to combat erectile dysfunction. Fatigue is an important component of sexual dysfunction. It was evaluated the anti-fatigue effect of sildenafil using forced swim test in the literature but unfortunately sildenafil did not find to be possessed any anti-fatigue effect (66).

Treatment of alopecia areata

Alopecia areata is an acquired skin disease that can affect all hair-bearing skin and is characterized by localized areas of non-scarring hair loss. Alopecia areata is occasionally associated with any other external or internal medical problems. Most often these bald areas regrow their hair spontaneously. Alopecia areata is an

immunologically mediated cessation of hair growth primarily involving, but not limited to, the scalp. The treatment of alopecia areata involves promotion of hair growth (for instance with topical minoxidil application), immunosuppression (intralesional or systemic steroid therapy, phototherapy) or immunomodulation (anthralin, dinitrochlorobenzene, diphenylcyclopropenone, squaric acid dibutylester) (100). All these medications have some disadvantages and difficulties for the treatment of children with alopecia areata. The efficacy of sildenafil in children diagnosed with alopecia areata has been tested in an open-pilot study (100). Eight patients with (<= 25% of scalp surface area involvement) alopecia areata who were refractory to previous topical treatments applied 1% sildenafil twice daily for 3 months. All the patients completed the study. As a result two patients experienced vellus-type hair growth and one patient had terminal hair growth. However, these outcomes were accepted as the spontaneous regression of the disease. The use of topical 1% sildenafil for the treatment of alopecia areata without further evidence of its therapeutic benefit was not recommended (100).

Pros and cons of drug repurposing

In this review, it was provided an overview of drug repositioning for some drug and sildenafil. Several successful case studies were presented. Many of these drugs are still under investigation. Although drug repurposing should significantly reduce the time and cost associated with drug development processes, benefits are still limited to a certain process from preclinical to Phase studies. Many challenges still exist after Phase trials. Phase III studies involves much larger number of patients compared to Phase I and II studies. Due to the size and relatively long duration, Phase III studies are the most expensive and time-consuming trials and these hurdles in Phase III studies have not changed over the years (101). Another challenge that should be considered for drug repositioning has to do with intellectual property protection of the repurposed drugs, especially for those drugs that are off patents. Off-patent drugs can be protected in part by method-of-use patents which contain one or more claims directed to a method of use. These patents are much weaker than the composition-of-matter patents in terms of the exclusionary right.

Approved drugs keep accumulating over the years; on average 20 to 30 new molecules have been approved by FDA (102) each year and since more diverse and selective drug targets are being discovered and developed, the approved drug collections will be particularly useful to quickly identify clinically advanced and more effective drugs against those targets. A major problem of conventional drugs for instance cancer chemotherapy drugs are notorious side effects that significantly reduces the quality of life of patients. As most of the non-cancer drugs have little or tolerable side effects in humans, repurposing alone or in combination of them with other present drugs can help to reduce some undesirable side effects. The other big problem seems to be complicated mechanism. Many times clear and feasible pathway or mechanism

cannot be obtained or explained with repurposed drugs.

Conclusion

The ultimate goal of drug studies is to approve drugs that are safe and highly effective, ultimately improving care for the patient population can be obtained by developed molecules. Significant progress has been made in discovering new molecules using number of techniques such as combinatorial drug design or docking studies but these studies are still very expensive and time consuming. New clinical trials for known molecules for another indication, improving the environment for developing and conducting randomized clinical trials to find some new benefits of known drug molecules have been also developed over the years. As we learn a lot from sildenafil and clinical trials especially from pulmonary hypertension and adaptation it to pediatric population; all these challenges highlight that safety, efficacy and labeling for especially patients should be studied and established very well. There is still a general need to find some new usage for known molecules to reduce some costs and overcome some hurdles. Some new studies, literature searches should be performed but professional societies and regulators should also be partnering in these efforts.

All performed experiments and some unpublished results indicate that drug re-purposing is still a good choice and there are still some more opportunities. Some new uses for enzyme inhibitors like Sildenafil are still waiting to be discovered and it seems that all these results will be in the literature in near future.

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