Computational drug repositioning for peripheral arterial disease: prediction of anti-inflammatory and pro-angiogenic therapeutics

Liang-Hui Chu1*, Brian H. Annex2 and Aleksander S. Popel1

1 Department of Biomedical Engineering, School of Medicine, Johns Hopkins University, Baltimore, MD, USA, 2 Division of Cardiovascular Medicine, Department of Medicine and Robert M. Berne Cardiovascular Research Center, University of Virginia School of Medicine, Charlottesville, VA, USA

Peripheral arterial disease (PAD) results from atherosclerosis that leads to blocked arteries and reduced blood flow, most commonly in the arteries of the legs. PAD clinical trials to induce angiogenesis to improve blood flow conducted in the last decade have not succeeded. We have recently constructed PADPIN, protein-protein interaction network (PIN) of PAD, and here we combine it with the drug-target relations to identify potential drug targets for PAD. Specifically, the proteins in the PADPIN were classified as belonging to the angiome, immunome, and arteriome, characterizing the processes of angiogenesis, immune response/inflammation, and arteriogenesis, respectively. Using the network-based approach we predict the candidate drugs for repositioning that have potential applications to PAD. By compiling the drug information in two drug databases DrugBank and PharmGKB, we predict FDA-approved drugs whose targets are the proteins annotated as anti-angiogenic and pro-inflammatory, respectively. Examples of pro-angiogenic drugs are carvedilol and urokinase. Examples of anti-inflammatory drugs are ACE inhibitors and maraviroc. This is the first computational drug repositioning study for PAD.

Keywords: peripheral arterial disease, computational drug repositioning, inflammation, angiogenesis, drug-target network, bioinformatics, cardiovascular disease

Introduction

Recent pharmaceutical research and development (R&D) reports show that the probability of success for a new pharmaceutical compound to get to the market has declined in the last 10 years (Pammolli et al., 2011). The average time of drug development has increased from 9.7 years during the 1990s to 13.9 years from 2000 onwards. The average probability of success of total numbers of R&D projects in the cardiovascular system is only 4.86%. Drug repositioning, new use of old drugs, can shorten the development time and provide solutions for the high cost and declined number of new successful drugs of the pharmaceutical companies (Dudley et al., 2011). Computational repositioning strategies can predict new therapeutic indications for FDA-approved drugs, which then have to undergo clinical trials for the new indication (Belch et al., 2003; Ostchega et al., 2007; Shameer et al., 2015). In this study, we primarily use the network-based approach in computational drug repositioning.
Peripheral arterial disease (PAD) results from atherosclerosis, the plaque built-up inside the arteries, which blocks the blood flow in the peripheral arteries and most commonly in the arteries that perfuse the legs (Belch et al., 2003; Annex, 2013). Age, diabetes, and cigarette smoking are the major risk factors for the development of PAD (Belch et al., 2003; Ostchega et al., 2007; Annex, 2013). There are 8–12 million people with PAD in the United States (Writing Group et al., 2010). The clinical manifestations of PAD range from patients who do not report leg pain but have a lower functional capacity (approximately 50% of all PAD subjects) to patients who have intermittent claudication manifested as leg pain with walking/exercise that is relieved with rest (approximately 33–40% of all PAD subjects) (Hirsch et al., 2006; Norgren et al., 2007). With the goal to increase blood flow around blockages, clinical trials using drugs and gene delivery for therapeutic angiogenesis such as VEGF (vascular endothelial growth factor) gene delivery have been performed for the last two decades but have not been successful.

Hoier et al. showed that there was no difference in basal skeletal muscle VEGF mRNA content before and after passive or active exercise between PAD patients and control (Hoier et al., 2013). However, the basal level of anti-angiogenic protein thrombospondin-1 (TSP1) was remarkably higher in the PAD patients than control groups. They conclude that the anti-angiogenic factors dominate the pro-angiogenic factors in PAD patients. The up-regulation of TSP1 has been shown in various gene expression microarray studies of mouse (Chu et al., 2015) and human samples of PAD (Fu et al., 2008; Masud et al., 2012). Currently there are no FDA-approved drugs targeting TSP1. Therefore, the computational drug repositioning approach to predict the drugs targeting other endogenous anti-angiogenic proteins should be helpful for designing clinical trials for therapeutic angiogenesis in PAD.

Inflammation plays an important role in initiation and progression of PAD, and many circulating biomarkers such as matrix metalloproteinases (MMPs) and interleukin are considered as the clinical manifestation of PAD (Signorelli et al., 2014). Atherosclerosis is the dominant cause of many cardiovascular diseases, including myocardial infarction, heart failure, coronary artery disease (CAD), and stroke (Frostegård, 2013). Atherosclerosis is a chronic inflammatory condition. Potential anti-inflammatory treatments in atherosclerosis are reviewed in Frostegård (2013). The interplay between inflammation and endothelial progenitor cells is critical in cardiovascular diseases (Grisar et al., 2011). Combination of anti-inflammatory and pro-angiogenic treatments for PAD was suggested and validated in vivo by Zachman et al. (2014). However, a systematic bioinformatics approach to identify the potential drug repositioning for inhibition of anti-angiogenic and pro-inflammatory proteins for PAD is still lacking.

We previously constructed the PADPIN, protein-protein interaction network (PIN) in PAD that includes angiome, immunome, and arteriome, characterizing the processes of angiogenesis, immune response/infammation and arteriogenesis, respectively (Chu et al., 2015). We have analyzed several available microarray gene expression datasets from ischemic and non-ischemic muscles in two mouse models of PAD (in C57BL/6 and BALB/c mouse species) from Hazarika et al. (2013) to identify important genes/proteins in PAD, such as THBS1 (thrombospondin-1), TLR4 (toll-like receptor 4), EphA4 (EPH receptor A4), and TSPAN7 (tetraspanin 7). However, none of the four genes (THBS1, TLR4, EphA4, and TSPAN7) have FDA-approved drugs to target them. Considering the time (>10 years) and cost (> $1 billion) for developing a new drug agent, drug repositioning in PAD offers promise of providing effective therapeutics in shorter time and at lower cost compared to conventional de-novo drug discovery and development. In addition, drug repurposing is an approach of taking agents in development that have achieved adequate safety for one indication but are tested for efficacy in another when safety is already evident.

Materials and Methods

Resources for Drugs and Drug-target Interactions

We rely on two major resources for drug information and drug-target, DrugBank 3.0 http://www.drugbank.ca/ (Knox et al., 2011) and Pharmacogenomics Knowledge Base (PharmGKB) http://www.pharmgkb.org/ (Whirl-Carrillo et al., 2012). DrugBank contains extensive omics data, such as pharmacogenomic, pharmacoproteomic, and pharmacometabolomic data. We use DTome (Drug-Target interactome tool) (Sun et al., 2012) to compile all the drugs included in DrugBank 3.0 (Knox et al., 2011), including the approved, experimental, nutraceutical, illicit, and withdrawn drugs. We compile three binary relations in DrugBank from DTome: drug-drug, drug-gene, and drug-target interactions. This compilation provides the rich resources for the potential repositioning or repurposing. By considering the drug safety and development time, we focus on FDA-approved drugs in this study. We compiled the three binary relations from PharmGKB: gene-disease, gene-drug, and gene-gene interactions. The drug-target interactions were compiled from both DrugBank (Knox et al., 2011) and PharmGKB (Whirl-Carrillo et al., 2012).

Proteins in PADPIN and Therapeutic Angiogenesis in PAD

Details of the construction of PADPIN, protein-protein interaction (PIN) of PAD in angiogenesis, immune response and arteriogenesis, are described in Chu et al. (2015). The methodology is similar to that used for constructing the global PIN of angiogenesis (angiome) that comprises 1233 proteins and 5726 interactions (Chu et al., 2012). The PIN of immune response (immunome) comprises 3490 proteins and 21,164 interactions. The PIN of arteriogenesis (arteriome) comprises 289 proteins and 803 interactions. The degree of node represents the number of links to a node in the network. The network parameter was calculated by NetworkAnalyzer (Assenov et al., 2008) in Cytoscape (Smoot et al., 2011). We start with the genes listed in the three PINs, to find the interactive drugs from the DrugBank and PharmGKB. Note that in bioinformatics publications, and specifically in protein-protein networks

Frontiers in Pharmacology | www.frontiersin.org 2 August 2015 | Volume 6 | Article 179
publications, the terms “gene” and “protein” are sometimes used interchangeably; while we mostly use “protein” term in this context, we sometime use “gene” to be consistent with previous publications.

List of Anti-angiogenic and Pro-inflammatory Genes

The activation of a specific biological process can be implemented using two strategies. One is direct activation of the genes involved in positive regulation of that biological process; the other is inhibition of the genes involved in negative regulation of that biological process. Specifically for PAD, to stimulate vascular growth and remodeling and increase the blood flow, we propose inhibition of genes annotated as negative regulation of angiogenesis as a therapeutic approach to stimulating angiogenesis. The rationale for this approach is that numerous clinical trials aimed at stimulating angiogenesis by growth factors such as VEGF-A and FGF-2 have not been successful. We identified 39 anti-angiogenic genes, chosen by Gene Ontology (GO: 0016525) and literature (Chu et al., 2014). The endothelial dysfunction in patients with PAD is characterized by impaired nitric oxide signaling, excessive inflammation and diminished response to angiogenic factors (Annex, 2013). To inhibit the inflammation, we propose inhibition of pro-inflammatory responses as a therapeutic approach for anti-inflammatory treatment of PAD. There are 89 genes classified in positive regulation of inflammatory response (GO:0050729). We list these genes in Table 1.

Results

Drug-targets Relations in Angiome, Immunome and Arteriome of PADPIN

We collected 11,043 binary relations between the drug and drug targets from DrugBank 3.0 (Knox et al., 2011) and 3138 binary relations between the drug and associated genes of that drug, which may not be the direct targets, from PharmGKB (Whirl-Carrillo et al., 2012). By matching the genes in angiome, immunome, and arteriome with the drug targets listed in the drug-gene binary relations from DrugBank and PharmGKB, we build the complete tables of genes and repositioning drugs (Tables S1–S3). Table S1 shows 409 and 174 drug targets listed in angiome for the drugs from DrugBank and PharmGKB, respectively. We select the genes with at least one drug targeting that gene in angiome, and skip the genes without any drug targets. There might be multiple drugs targeting the same drug target; we list the multiple drugs in the same row of the table. Table S2 shows 865 and 382 drug targets in immunome for the drugs from DrugBank and PharmGKB, respectively. Table S3 shows 82 and 46 drug targets in arteriome for the drugs from DrugBank and PharmGKB, respectively.

We rank the genes in angiome, immunome, and arteriome by the degree of nodes, i.e., number of links of the nodes in the network, in Tables S1–S3, respectively. Tables S1–S3 provide the complete list of drugs and drug targets which are annotated in angiogenesis, immune response/inflammation, and arteriogenesis. Tables S1–S3 provide the complete list of drugs in DrugBank and PharmGKB, including approved, experimental, nutraceutical, illicit, and withdrawn drugs. Considering the drug safety and efficacy issues, we mostly consider the FDA-approved drugs in the predictions of repositioning drugs (Table S4).

Inhibition of Anti-angiogenic Pro-inflammatory Genes

We postulate two strategies to the PAD treatment: pro-angiogenic and anti-inflammatory. Starting from the 39 genes annotated in negative regulation of angiogenesis (see Materials and Methods), we match the genes with drug targets and drugs listed in Table S1, and only list the FDA-approved drugs from DrugBank in Table 2. The five genes are CCL2, NPPB, NPR1, PF4, and SERPINE1. These drugs include mimosine targeting CCL2, carvedilol targeting NPPB, nitroprusside targeting NPR1, urokinase, reteplase, and drotrecogin alfa targeting SERPINE1. The beta-blockers (e.g., carvedilol in our prediction) in general have not been shown to affect PAD symptoms, but they do not make PAD symptoms worse (Paravastu et al., 2013). Infusion of recombinant-based plasminogen activator (e.g., reteplase) and urokinase can clear blood clots and restore blood flow.

### Table 1 | List of 39 anti-angiogenic and 89 pro-inflammatory genes.

| Categories                | Gene ontology                          | List of genes                                                                 |
|---------------------------|----------------------------------------|--------------------------------------------------------------------------------|
| Anti-angiogenic            | Negative regulation of angiogenesis    | AMOT, ANGPT2, APOH, BA1, CCL2, CCR2, COL4A2, COL4A3, CXCL10, FASLG, FOXO4, GHR, GF21, HDAC5, HHEX, HOXA5, HRG, KL4, KLF3, KRT11, LECT1, LIF, MAP2K5, NF1, NPPB, NPR1, PF4, PML, PTPRM, ROCK1, ROCK2, SERPINE1, SERPINF1, STAB1, THBS1, THBS2, THBS4, TIE1 |
| Pro-inflammatory          | Positive regulation of inflammatory response | ACE, ADAM8, ADORA2B, ADORA3, AGER, AGT, AGTR1, ALOX5AP, AOC3, C3, CCL24, CCL3, CCL3L3, CCL5, CCR5, CXCL12, CD28, CD47, CLOCK, CTSS, CX3CL1, EDNRA, EGFR, FABP4, FCER1A, FCEG1R, FCGRI1A, FCGRI2A, FFAR3, GPRC5B, HISP1, HYAL2, IDO1, IL1B, IL1B, IL1B, IL1B, IL1B1L1, IL2, IL2, IL23A, IL33, IL6, IL6ST, ITGAA2, JAK2, LBP, LTA, MAPK13, MIF, NLRP12, NPVR, OSM, OSMR, PDE2A, PDE5A, PIK3CG, PLA2G2A, PLA2G4A, PLA2G7, PRKCA, PTGER3, PTGER4, PTGS2, RPS19, S100A12, S100A8, S100A9, SERPINE1, STAT5A, STAT5B, TAC1, TGM2, TL1A, TL1B, TL1A, TL1B, TL1B, TNF, TNFRSF11A, TNFRSF11B, TNFRSF11C, TNFRSF11D, TNFRSF11E, TNFRSF11F, TNFRSF11G, TNFRSF11H, TNFRSF11I, TNFRSF11J, TNFRSF11K, TNFRSF11L, TNFRSF11M, TNFRSF11N, TNFRSF11O, TNFRSF11P, TNFRSF11Q, TNFRSF11R, TNFRSF11S, TNFRSF11T, TNFRSF11U, TNFRSF11V, TNFRSF11W, TNFRSF11X, TNFRSF11Y, TNFRSF11Z, ZP3 |
TABLE 2 | Predictions of pro-angiogenic FDA-approved drugs that target anti-angiogenic genes.

| Gene symbol | Gene name | DrugBank | Physiological relevance in PAD or CAD |
|-------------|-----------|----------|--------------------------------------|
| CCL2        | Chemokine (C-C motif) ligand 2 | Mimosine, danazol | Potential indicator of atherosclerosis in PAD (Rull et al., 2011) |
| NPPB        | Natriuretic peptide B | Carvediol | Three SNPs at NPPB locus associated with lower risk of PAD (Hu et al., 2013) |
| NPR1        | Natriuretic peptide receptor 1 | Nitroprusside, nitroglycerin, isosorbide dinitrate, amyl nitrite, erythryl tetranitrate, nesiritide | PF4 level increasing in patients with coronary artery ectasia (Yasar et al., 2007) |
| PF4         | Platelet factor 4 | Drotrecogin alfa | Plasminogen activator inhibitor-1 (PAI-1) increasing in patients with CLI (critical limb ischemia), leading to prothrombotic (Björck et al., 2013) |
| SERPINE1    | Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 | Alteplase, urokinase, reteplase, anistreplase, tenecteplase, drotrecogin alfa | |

Inhibition of Pro-inflammatory Genes

We match the 89 pro-inflammatory genes with drug targets and drugs listed in Table S2, and only list the FDA-approved drugs from DrugBank in Table 3 (see the list of pro-inflammatory genes in Methods). The corresponding FDA-approved drugs include maraviroc (an antiretroviral drug, a CCR5 inhibitor), bosentan (a dual endothelin receptor antagonist that affects both endothelin A and B receptors, used in the treatment of pulmonary artery hypertension), sitaxentan (endothelin A receptor antagonist, used in the treatment of pulmonary artery hypertension), cetuximab (EGFR antagonist, used in several types of cancer) and imiquimod (an immune response modulator, used for skin diseases including skin cancer).

To find the physiological relevance of these pro-inflammatory genes in PAD, we continue to use PubMed to find the relevant references. References in Table 3 support our hypothesis that anti-inflammatory drugs have high potential for repositioning for PAD. Some drugs cannot improve ABI (ankle-pressure index) of PAD patients but can improve the walking ability in patients with critical limb ischemia (CLI), such as ACE inhibitors (Hunter et al., 2013; Shahin et al., 2013). Some genes are indicated as related with PAD, such as C3 (complement component 3) (Fehervari et al., 2014), PTGS2 (prostaglandin-endoperoxide synthase 2) (Flórez et al., 2009), SERPINE1 (Björck et al., 2013), S100A12 (Shiotsu et al., 2011), and TNF (Botti et al., 2012; Wozniak et al., 2012; Gardner et al., 2014). Some genes are potential biomarkers or associated with other cardiovascular diseases, such as AGTR1 (angiotensin II receptor, type 1) in coronary occlusive disease (Baños et al., 2011), CCR5 in pulmonary arterial hypertension (Amsellem et al., 2014), LTA (lymphotoxin alpha) in CAD (Topol et al., 2006), and PRKCA (protein kinase C, alpha) in atherosclerosis (Konopatskaya and Poole, 2010). Many of the anti-inflammatory genes in Table 3 are not directly associated with PAD or CAD based on PubMed search, such as ADORA2B (adenosine A2b receptor), EDNRA (endothelin receptor type A), FCER1G (Fc receptor, IgE, high affinity I, gamma polypeptide), STAT5B (signal transducer and activator of transcription 5B), and TLR9 (toll-like receptor 9). In general, the physiological evidence of these anti-inflammatory genes listed in Table 3 strongly supports our hypothesis that inhibition of pro-inflammatory genes is a viable drug repositioning strategy in PAD.

Visualization of Drug-target Network

Graph representation is used to visualize pro-angiogenic and anti-inflammatory repositioning drugs for PAD in Figures 1, 2, respectively. We plot the drug-target networks of the anti-angiogenic and pro-inflammatory proteins for the drugs in Tables 2, 3, respectively. We represent the drug target by pink circle and the drug by blue square. Figure 1 shows several compounds targeting the proteins which are annotated as negative regulation of angiogenesis. Figure 2 shows the drug-target networks of the anti-angiogenic drugs and targets from Table 3. The number of inflammation targets and drugs in Figure 2 is much larger than anti-angiogenic targets and drugs in Figure 1. This gives the insight for the development of clinical trials of anti-inflammatory drugs in PAD in the future. We will discuss the potential clinical trials in Discussion.

Discussion

The clinical trials aimed at stimulating VEGF in PAD and CAD have been unsuccessful (Annex, 2013). The exercise therapy has been demonstrated as the beneficial treatment...
| Gene symbol | Description                               | DrugBank                                                                 | Physiological relevance in PAD or CAD                                                                 |
|------------|-------------------------------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| ACE        | Angiotensin I converting enzyme           | Ramipril, fosinopril, trandolapril, benazepril, enalapril, candesartan,   | ACE inhibitor helping the walking ability in patients with CLI, but not improving ABI (ankle-pressure index) (Hunter et al., 2013; Shahin et al., 2013) |
|            |                                           | moexipril, lisinopril, enalapril, quinapril, rescinnamine, captopril,      |                                                                                                      |
|            |                                           | cilazapril, spirapril                                                    |                                                                                                      |
| ADORA2B    | Adenosine A2b receptor                     | Theophylline, adenosine, enprofylline, defibrotide                       |                                                                                                      |
| AGTR1      | Angiotensin II receptor, type 1           | Valsartan, olmesartan, losartan, candesartan, eprosartan, telmisartan,  | Correlation between the increased AGTR1 and cardiovascular risk factors (Bahos et al., 2011)           |
|            |                                           | irbesartan, forasartan, sarpisartan, tasosartan                          |                                                                                                      |
| AOC3       | Amine oxidase, copper containing 3         | Phenelzine, hydralazine                                                 |                                                                                                      |
| C3         | Complement component 3                    | Intravenous immunoglobulin                                              | C3 level in serum associated with ABI and atherosclerosis in PAD patients (Fehervari et al., 2014)     |
| CCR5       | Chemokine (C-C motif) receptor 5           | Maraviroc                                                              | A treatment target in pulmonary arterial hypertension (Amsellem et al., 2014)                         |
| CNR1       | Cannabinoid receptor 1 (brain)             | Dronabinol, nabrone, rimonabant, dronabinol                              |                                                                                                      |
| EDNRA      | Endothelin receptor type A                 | Bosentan, sitaxentan                                                   |                                                                                                      |
| EGFR       | Epidermal growth factor receptor           | Cetuximab, trastuzumab, lidocaine, gefitinib, erlotinib, lapatinib,       |                                                                                                      |
|            |                                           | panitumumab                                                             |                                                                                                      |
| FCER1A     | Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide | Omalizumab, benzylpenicilloyl pollysine                                 |                                                                                                      |
| FCER1G     | Fc receptor, IgE, high affinity I, gamma polypeptide | Benzylpenicilloyl pollysine                                             |                                                                                                      |
| FCGR1A     | Fc fragment of IgG, high affinity Ia, receptor (CD64) | Cetuximab, etanercept, intravenous immunoglobulin, adalimumab, abciximab,   |                                                                                                      |
|            |                                           | gemtuzumab ozogamicin, trastuzumab, rituximab, basiliximab, muromonab,   |                                                                                                      |
|            |                                           | ibritumomab, tositumomab, alemtuzumab, alefacect, efalizumab,             |                                                                                                      |
|            |                                           | natalizumab, palivizumab, daclizumab, bevacizumab, porflimer             |                                                                                                      |
| FCGR2A     | Fc fragment of IgG, low affinity Ila, receptor (CD32) | Cetuximab, etanercept, intravenous immunoglobulin, adalimumab, abciximab,   |                                                                                                      |
|            |                                           | gemtuzumab ozogamicin, trastuzumab, rituximab, basiliximab, muromonab,   |                                                                                                      |
|            |                                           | ibritumomab, tositumomab, alemtuzumab, alefacect, efalizumab,             |                                                                                                      |
|            |                                           | natalizumab, palivizumab, daclizumab, bevacizumab                        |                                                                                                      |
| IL1B       | Interleukin 1, beta                        | Minocycline, gallium nitrate, canakinumab                              | Phase III clinical trial targeting IL-6 by tocilizumab in cardiovascular disease (Ridker and Lüscher, 2014) |
| IL6        | Interleukin 6                              | Ginseng                                                                 | Implicated in predisposition for heart attack by genome-wide association studies (GWAS) (Topol et al., 2006) |
| LTA        | Lymphotixin alpha                          | Etanercept                                                              |                                                                                                      |

(Continued)
TABLE 3 | Continued

| Gene symbol | Description | DrugBank | Physiological relevance in PAD or CAD |
|-------------|-------------|----------|--------------------------------------|
| PDE5A | phosphodiesterase 5A, cGMP-specific | Sildenafil, theophylline, pentoxifylline, tadalafil, vardenafil, dipyridamole, udenafil | PDE5 inhibition promotes ischemia-induced angiogenesis (Sahara et al., 2010) |
| PLA2G2A | Phospholipase A2, group IIa (platelets, synovial fluid) | Indomethacin, diclofenac, ginkgo biloba, suramin, ginkgo biloba | |
| PLA2G4A | Phospholipase A2, group IVa (cytosolic, calcium-dependent) | Fluticasone propionate, quinacrine | |
| PRKCA | Protein kinase C, alpha | Phosphatidylserine, vitamin E | The key protein in regulation of platelet function and thrombosis in arteries (Konopatskaya and Poole, 2010) |
| PTGER3 | Prostaglandin E receptor 3 (subtype EP3) | Bimatoprost, dinoprostone, misoprostol | |
| PTGS2 | Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) | Gamma-homolinoic acid, icosapent, amsalicylic acid, mesalazine, acetylsalphen, indomethacin, nabumetone, ketorolac, tenoxicam, lenalidomide, celecoxib, tolmetin, piroxican, fenoprofen, diclofenac, sulindac, flurbiprofen, etodolac, mefenamic acid, naproxen, sulfasalazone, phenylbutazone, meloxicam, carprofen, difunisal, suprofen, salicylic acid, meclofenamic acid, acetylsalicylic acid, bromfenac, oxaprozin, ketoprofen, balsalazide, thalidomide, ibuprofen, lumaritoxib, magnesium salycilate, salicylate-sodium, salsalate, trisalicylate-choline, ginseng, antrafenine, antipyrine, tapirofenic acid, etoricoxib, riflunic acid, iomoxicam, nepafenac, gamma-homolinoic acid, icosapent, ginseng, thalidomide | PTGS2 (COX2) inhibition improves inflammation and endothelial dysfunction in PAD patients with intermittent claudication (IC) (Florez et al., 2009) |
| S100A12 | S100 calcium binding protein A12 | Olopatadine, amlexanox | The potential biomarker for chronic arterial disease (Sato et al., 2012) and associated with PAD (Shiotsu et al., 2011) |
| SERPINE1 | Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 | Alteplase, urokinase, reteplase, anistreplase, tenecteplase, drotrecogin alfa | Level of plasminogen activator inhibitor-1 (PAI-1) increased in patients with CII (critical limb ischemia) (Blörck et al., 2013) |
| STAT5B | Signal transducer and activator of transcription SB | Dasatinib | |
| TLR2 | Toll-like receptor 2 | Ospla lipoprotein | TLR2 and TLR4 expression increase during atherosclerosis, but only TLR4 gene expression associated with PAD (Varela et al., 2015) |
| TLR7 | Toll-like receptor 7 | Imiquimod, hydroxychloroquine | |
| TLR9 | Toll-like receptor 9 | Chloroquine, hydroxychloroquine | |
| TNF | Tumor necrosis factor | Etanercept, adalimumab, infliximab, chloroquine, thalidomide, glucosamine, clenbuterol, pranukast, amrinone, thalidomide | Circulating TNF higher in PAD patients than control (Gardner et al., 2014); Circulating cytokines induce endothelial dysfunction in PAD patients (Botti et al., 2012); Negative correlation between TNF concentration and pain free walking distance (Wozniak et al., 2012) |
| TNFSF11 | Tumor necrosis factor (ligand) superfamily, member 11 | Lenalidomide | |
for PAD, including walking tolerance, modified inflammatory markers, and adaptation of the limb (e.g., angiogenesis and arteriogenesis) (Haas et al., 2017). Clinical trials with agents targeting angiogenesis and inflammation, other than stimulation of VEGF, should be considered in the future. Below we provide insights for the potential repositioning drugs in PAD identified in this study, including the mechanism of action of these drugs, case studies for several selected drugs in clinical trials, and future experimental validations.

**Mechanism of Action of Repositioning Drugs for PAD**

Tables 1, 2 provide the anti-angiogenic and pro-inflammatory genes/proteins, drugs targeting these molecules, and physiological evidence for the involvement of these molecules in PAD. However, even though these drug-targets have been identified by our bioinformatics approaches, the mechanism of action of these drugs in PAD and the feasibility of the clinical trials need to be elucidated. Specifically, the effect of some of these drugs to promote angiogenesis in PAD by targeting anti-angiogenic proteins is unknown. Therefore, we search PubMed for the drugs listed in Table 2 using the keywords “(drug name) AND angiogenesis” to understand the mechanism and original use of these putative pro-angiogenic drugs. We list the drugs with at least one supporting reference found in PubMed in Table 4. These drugs include beta-1 adrenergic receptors blocker (carvedilol, targeting NPPB), vasodilator (isosorbide dinitrate, targeting
et al. (2010) examined plasma levels of endothelin and showed that endothelin was measured by laser Doppler perfusion imaging. de Haro Miralles et al. (2000) demonstrated that endothelin, antagonists, bosentan, and darusentan (LU13525) increased tissue blood flow in the extremities in patients with PAD. In a pre-clinical PAD model, Luyt et al. (2000) showed that endothelin could improve perfusion to the lower extremities compared to non-PAD controls. Just as importantly, patients with the most severe form of PAD, CLI, did not demonstrate elevated levels of endothelin, which suggests that an elevation of endothelin is specific to the pathophysiology of intermittent claudication and not all forms of PAD. The original indication of zibotentan was in oncology and pulmonary artery hypertension. The reuse of an endothelin receptor antagonists in PAD patients with intermittent claudication is now in a Phase II clinical trial; the details of the clinical trial of zibotentan are provided in ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT01890135?term=NCT01890135&rank=1.

Case II: Carvedilol Targeting NPPB
Carvedilol has anti-inflammatory and pro-angiogenic effects in chronic ischemic cardiomyopathy (Le et al., 2013). Carvedilol showed improvement of myocardial flow and reduction of inflammation in the canine model of multivessel cardiomyopathy. The anti-inflammatory cytokine IL-10, which inhibits inflammatory cytokines such as TNFα, IL-1, IL-6, IL-8, and IL-12, was up-regulated in the carvedilol-treated animals. In the PAD microarray data, the inflammatory cytokine IL-8 was up-regulated as found in Masud et al. (2012) and Croner et al. (2012). Though beta-blockers are commonly used in patients with PAD, currently there are no specific clinical trials for carvedilol being compared to placebo or other beta-blockers in PAD patients.

Case III: Maraviroc Targeting CCR5
Maraviroc is an HIV drug targeting CCR5, which is involved in the inflammation pathway (Francisci et al., 2014). Therefore, maraviroc could have anti-inflammatory and anti-atherosclerosis effects, and become a potential repositioning drug in PAD. Croner et al. (2012) show the up-regulation of CCR5 in microarrays from the human femoral artery in PAD. CCR5 inhibitor maraviroc also blocks cell migration and metastasis, but not directly affects the angiogenesis pathway in triple negative breast cancer cell lines (Lee et al., 2014). Currently there are no clinical trials for maraviroc in PAD patients.

Limitations of Computational Drug Repositioning Approaches
There are several limitations by the computational approaches to predict the repositioning drugs in PAD. First, PAD is a...
TABLE 5 | Mechanism of Action and original use of the drugs for repositioning as anti-inflammatory in PAD.

| Drug name       | Target in inflammation | Degree of target in immunome | Mechanism of action                        | Original use                                      | PubMed search                      |
|-----------------|------------------------|------------------------------|--------------------------------------------|--------------------------------------------------|------------------------------------|
| Lapatinib       | EGFR                   | 137                          | Tyrosine kinase inhibitor                  | Breast cancer                                    | Hall et al., 2009                  |
| Lidocaine       | EGFR                   | 137                          | Stopping nerves from sending pain signal   | Local anesthetic and class-1b antimythic drug     | Caracas et al., 2009               |
| Masaviroc       | CCR5                   | 46                           | CCR5 receptor antagonist class             | Human Immunodeficiency virus (HIV) infection      | Francischi et al., 2014            |
| Dasatinib       | STAT5B                 | 33                           | Bcr-Abl tyrosine kinase inhibitor          | Leukemia                                         | Futosi et al., 2012                |
| Chloroquine     | TNF, TLR9              | 22, 22                       | 4-aminoquinoline drug                     | Malaria, rheumatoid arthritis                    | Yang et al., 2013                  |
| Celnbuterol     | TNF                    | 22                           | Angiotensin-converting enzyme (ACE) inhibitor | Hypertension and heart failure                   | Cudmore et al., 2013               |
| Glucosamine     | TNF                    | 22                           | Endogenous amino-monosaccharide synthesized from glucose | Promoting joint and cartilage health             | Azuma et al., 2015; Chou et al., 2015 |
| Infliximab      | TNF                    | 22                           | Chimeric monoclonal antibody against TNF alpha | Rheumatoid arthritis, psoriatic arthritis, ankyllosing spondyilitis | Hirono et al., 2009               |
| Aleplase        | SERPINE1               | 18                           | Plasminogen activator                     | Acute ischemic stroke                            | Lapchak and Araujo, 2007; Hacke et al., 2008 |
| Intravenous immunoglobulin | C3, FCGR1A, FCGR2A | 30, 13, 11 | IgG antibodies                              | Immune deficiencies, autoimmune diseases        | Nimmerjahn and Ravetch, 2008       |
| Drotrecogin alfa | SERPINE1               | 18                           | Recombinant form of human activated protein C | Decrease inflammation and the formation of blood-clots in blood vessels | Rice and Bernard, 2004 |
| Candesartan     | AGTR1                  | 16                           | Angiotensin II receptor antagonist         | High blood pressure and heart failure            | Yu et al., 2007                    |
| Eprosartan      | AGTR1                  | 16                           | Angiotensin II receptor antagonist         | Treats high blood pressure                      | Rahman et al., 2002                |
| Irbesartan      | AGTR1                  | 16                           | Angiotensin II receptor blocker (ARB)      | High blood pressure                             | Taguchi et al., 2013               |
| Losartan        | AGTR1                  | 16                           | Angiotensin II receptor blocker (ARB)      | High blood pressure                             | Merino et al., 2012                |
| Olmesartan      | AGTR1                  | 16                           | Angiotensin II receptor blocker (ARB)      | High blood pressure                             | Nagib et al., 2013                 |
| Telmisartan     | AGTR1                  | 16                           | Angiotensin II receptor blocker (ARB)      | High blood pressure                             | Al-Heijaj et al., 2011             |
| Valsartan       | AGTR1                  | 16                           | Angiotensin II receptor blocker (ARB)      | High blood pressure                             | Wang et al., 2014                  |
| Adalimumab      | FCGR1A, FCGR2A, TNF    | 13, 11, 22                   | Monoclonal antibody against TNF-alpha      | Arthritis, ankyllosing spondyilitis              | Jiang et al., 2013                 |
| Lenalidomide    | TNFSF11                | 15                           | Immunomodulatory and antiangiogenic agent  | Anemia and multiple myeloma                      | Rozovski et al., 2013              |
| Thalidomide     | PTGS2, TNF             | 7, 22                        | Immunomodulatory drug                      | Certain cancers, leprosy                         | Keiler et al., 2001                |
| Etanercept      | FCGR1A, FCGR2A, LTA, TNF | 13, 11, 7, 22             | Tumor necrosis factor (TNF) inhibitor      | Rheumatoid arthritis, psoriatic arthritis, ankyllosing spondyilitis, and plaque psoriasis | Caore et al., 2015                 |
| Abciximab       | FCGR1A, FCGR2A         | 13, 11                       | Inhibits platelet aggregation by preventing the binding of fibrinogen | Patients undergoing percutaneous coronary intervention (PCI) | Hong et al., 2007                  |
| Alefacept       | FCGR1A, FCGR2A         | 13, 11                       | Immune suppressant                         | Control of inflammation in moderate to severe psoriasis | Kraan et al., 2002; Chami et al., 2005 |
| Alemtuzumab     | FCGR1A, FCGR2A         | 13, 11                       | Binds to CD52, a protein present on the surface of mature lymphocytes | Chronic lymphocytic leukemia, multiple sclerosis | Helm et al., 2013                  |
| Daclizumab      | FCGR1A, FCGR2A         | 13, 11                       | Monoclonal antibody binding to CD25, alpha subunit of the IL-2 receptor of T cells | Prevents rejection in organ transplantation, multiple sclerosis | Papalid et al., 2003               |
| Drug name     | Target in inflammation | Degree of target in immunome | Mechanism of action                                                                 | Original use                                         | PubMed search   |
|--------------|------------------------|-----------------------------|-------------------------------------------------------------------------------------|-----------------------------------------------------|-----------------|
| Efalizumab   | FCGR1A, FCGR2A         | 13, 11                      | Immunosuppressant by inhibiting lymphocyte activation                              | Psoriasis                                           | Pan et al., 2014|
| Rituximab    | FCGR1A, FCGR2A         | 13, 11                      | Monoclonal antibody against the protein CD20                                         | Rheumatoid arthritis                                | Baslund et al., 2012|
| Canakinumab  | IL1B                   | 10                          | Monoclonal antibody targeted at interleukin-1 beta                                 | Rheumatoid arthritis, coronary artery disease       | Ridker et al., 2011|
| Gallium nitrate | IL1B                | 10                          | Gallium salt of nitric acid                                                         | Symptomatic hypercalcinemia                         | Eby, 2005       |
| Minoxydil    | IL1B                   | 10                          | Bacteriostatic antibiotic                                                          | Treats infections                                   | Leite et al., 2011|
| Aminosalicylic acid | PTGS2          | 7                           | Inhibits folic acid synthesis and synthesis of the cell wall component             | Tuberculosis                                        | Williams et al., 2011|
| Balsalazide  | PTGS2                  | 7                           | Converted in the body to mesalamine and reducing bowel inflammation                | Ulcerative colitis                                  | Pardi et al., 2002|
| Acetaminophen| PTGS2                 | 7                           | Analgesics (pain relievers)                                                        | Treats minor aches and pain and reduces fever       | Jeon et al., 2014|
| Acetylsalicylic acid (aspirin) | PTGS2 | 7                           | Antiplatelet effect by inhibiting thromboxane                                       | Prevention of arterial and venous thrombosis        | Herová et al., 2014|
| Bromfenac    | PTGS2                  | 7                           | Non-steroidal anti-inflammatory drug (NSAID)                                        | Pain and swelling of the eye after cataract surgery | Raja et al., 2014|
| Etodolac     | PTGS2                  | 7                           | Non-steroidal anti-inflammatory drug (NSAID)                                        | Treats pain caused by arthritis                     | Costa et al., 2008|
| Etoricoxib   | PTGS2                  | 7                           | COX-2 selective inhibitor                                                          | Rheumatoid arthritis, psoriatic arthritis, osteoarthritis | Moraes et al., 2007|
| Gamma-homolinolenic acid | PTGS2 | 7                           | Omega-6 fatty acid                                                                | Dietary supplement for a variety of human health problems | Kapoor and Huang, 2006|
| Carprofen    | PTGS2                  | 7                           | Reduces inflammation by inhibition of COX-2                                         | Pain and inflammation from arthritis                | Fox and Johnston, 1997|
| Celecoxib    | PTGS2                  | 7                           | COX-2 selective non-steroidal anti-inflammatory drug (NSAID)                        | Treats pain caused by arthritis                     | Chen et al., 2008|
| Ibuprofen    | PTGS2                  | 7                           | Non-steroidal anti-inflammatory drug (NSAID)                                        | Pain and fever                                       | Chmiel et al., 2015|
| Ketoprofen   | PTGS2                  | 7                           | Non-steroidal anti-inflammatory drugs (NSAIDs)                                      | Relief of pain and inflammation such as in rheumatic disease | Choi et al., 2013|
| Ketorolac    | PTGS2                  | 7                           | Non-steroidal anti-inflammatory drugs (NSAIDs)                                      | Pain and inflammation caused by arthritis           | Donnenfeld et al., 2011|
| Lornoxicam   | PTGS2                  | 7                           | Non-steroidal anti-inflammatory drug                                                | Pain, especially resulting from inflammatory diseases | Buritova and Besson, 1997, 1998|
| Mefenamic acid | PTGS2               | 7                           | Non-steroidal anti-inflammatory drug (NSAID)                                        | Pain                                                 | Cunha et al., 2014|
| Meloxicam    | PTGS2                  | 7                           | Non-steroidal anti-inflammatory drug (NSAID)                                        | Pain                                                 |                 |
| Mesalazine   | PTGS2                  | 7                           | 5-amino-2-hydroxybenzoic acid                                                       | Inflammatory bowel disease, such as ulcerative colitis |                 |
| Nabumetone   | PTGS2                  | 7                           | Non-steroidal anti-inflammatory drug (NSAID)                                        | Relief of pain and inflammation in arthritis        |                 |
| Naproxen     | PTGS2                  | 7                           | Non-steroidal anti-inflammatory drugs (NSAIDs)                                      | Relief of pain, fever, swelling, and stiffness      |                 |

(Continued)
| Drug name   | Target in inflammation | Degree of target in immunome | Mechanism of action                                      | Original use                                                                 | PubMed search                  |
|------------|------------------------|-------------------------------|----------------------------------------------------------|------------------------------------------------------------------------------|--------------------------------|
| Nepafenac  | PTGS2                  | 7                             | Non-steroidal anti-inflammatory drugs (NSAIDs)            | Eye pain and swelling after cataract surgery                                 | Naedi et al., 2007            |
| Niflumic acid | PTGS2                | 7                             | Inhibitor of cyclooxygenase-2                            | Joint and muscular pain                                                     | Bieleon et al., 2003          |
| Piroxicam  | PTGS2                  | 7                             | Non-steroidal anti-inflammatory drugs (NSAIDs)           | Pain, including arthritis pain                                              |                               |
| Salsalate  | PTGS2                  | 7                             | Non-steroidal anti-inflammatory drugs (NSAIDs)           | Rheumatoid arthritis                                                        | Goldfine et al., 2008         |
| Sulindac   | PTGS2                  | 7                             | Non-steroidal anti-inflammatory drug (NSAID)             | Treats pain caused by arthritis, gout, or sore tendons                       | Mladenova et al., 2013        |
| Tenoxicam  | PTGS2                  | 7                             | Non-steroidal anti-inflammatory drugs (NSAID)            | Relieve inflammation and pain associated with rheumatoid arthritis          |                               |
| Indomethacin | PTGS2, PLA2G2A       | 7, 6                          | Non-steroidal anti-inflammatory drugs (NSAIDs)           | Reduce fever, pain, stiffness, and swelling                                  | Glaser et al., 1993           |
| Suramin    | PLA2G2A                | 6                             | Antimicrobial drug                                        | Protozoa, Helminthiasis                                                     | Shiono et al., 2002; Lu et al., 2012 |
| Bosentan   | EDNRA                  | 4                             | Dual endothelin receptor antagonist                       | Pulmonary artery hypertension                                               | Shetty and Derk, 2011         |
| Omalizumab | FCER1A                 | 3                             | Reduce sensitivity to inhaled or ingested allergens      | Ashma                                                                       | Holgate et al., 2005, 2009    |
| Enalapril  | ACE                    | 2                             | Angiotensin-converting enzyme (ACE) inhibitor           | High blood pressure                                                         | Yan et al., 2013              |
| Captopril  | ACE                    | 2                             | Angiotensin-converting enzyme (ACE) inhibitor           | High blood pressure and heart failure                                        | El Desoky, 2011               |
| Dinoprostone | PTGER3               | 2                             | Prostaglandin E2 (PGE2)                                  | Helps dilate the opening of the uterus ( cervix) in a pregnant woman         | Tang et al., 2012             |
| Dipyridamole | PDE5A                | 2                             | Inhibits the phosphodiesterase enzymes, coronary vasodilator | Inhibits thrombus formation                                                  | Weyrich et al., 2005; Massaro et al., 2013 |
| Enalapril  | ACE                    | 2                             | Angiotensin converting enzyme (ACE) inhibitors          | Treats high blood pressure                                                  | da Cunha et al., 2005         |
| Misoprostol | PTGER3                | 2                             | Non-steroidal anti-inflammatory drugs (NSAIDs)           | Prevents stomach ulcers                                                     | Rossetti et al., 1995         |
| Pentoxifylline | PDE5A               | 2                             | Phosphodiesterase inhibitor                              | Treating intermittent claudication resulting from peripheral artery disease  | Abdel-Salam et al., 2003; Fernandes et al., 2008                           |
| Perindopril | ACE                   | 2                             | ACE inhibitor                                            | Treating intermittent claudication resulting from peripheral artery disease  | Rajski et al., 2006           |
| Quinapril  | ACE                    | 2                             | ACE inhibitor                                            | High blood pressure and heart failure                                        | Egido and Ruiz-Ortega, 2007   |
| Tadalafil  | PDE5A                  | 2                             | Inhibiting cGMP-specific phosphodiesterase type 5       | Erectile dysfunction, pulmonary arterial hypertension                        | de Visser et al., 2009        |
| Theophylline | PDE5A, ADORA2B       | 2, 2                          | Methylxanthine drug                                      | Asystole, respiratory conditions                                             | Caso et al., 2009             |
| Vardenafil | PDE5A                  | 2                             | PDE5 inhibitor                                           | Erectile dysfunction                                                        | Lubamba et al., 2012          |
| Hydralazine | AOC3                   | 1                             | Vasodilator                                              | Treats high blood pressure and heart failure                                 | Bai et al., 2012              |
complex disease caused by many risk factors and classified by different stages of diseases. Our methods cannot predict the repositioning drugs based on various conditions in PAD patients. Second, the current available clinical trials based on these predicted repositioning drugs in PAD patients are very limited. The available gene expression dataset in human PAD and mouse PAD model is limited. It is difficult to validate our predictions by current clinical trials and available microarray data. Third, the pro-angiogenic and anti-inflammatory drug-target networks cannot directly link the drugs to PAD based on the current physiological evidence in PAD. The value of the computational drug repositioning might be limited for clinical trial design.

Conclusions

Our study provides comprehensive predictions of potential pro-angiogenic and anti-inflammatory drugs and drug targets for PAD patients. Based on the protein-protein interaction network PADPIN, we collected the binary relations between FDA-approved drugs and genes annotated in PADPIN. By gathering FDA-approved drugs, these predictions form a basis for further validation and future translational research in PAD.

Acknowledgments

LC, AP, and BA proposed the idea; LC implemented the simulation; LC, BA, and AP edited the paper. The research was supported by NIH grant HL101200 and R21 HL122721 (AP, BA) and 1UH3TR00959 (BA).

Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fphar.2015.00179

References

Abdel-Salam, O. M., Baioumy, A. R., El-Shenawy, S. M., and Arbid, M. S. (2003). The anti-inflammatory effects of the phosphodiesterase inhibitor pentoxifylline in the rat. Pharmacol. Res. 47, 331–340. doi: 10.1016/S1043-6618(03)0002-1
Albert, W. K., Numan, L. T., Al-Shaib, R. Z., and Hussain, S. A. (2011). Anti-inflammatory activity of telmisartan in rat models of experimentally-induced chronic inflammation: comparative study with dexamethasone. Saudi Pharm. J. 19, 29–34. doi: 10.1016/j.jsps.2010.10.004
Amsellem, V., Lipskaia, L., Abid, S., Poupel, L., Houssaini, A., Quarck, R., et al. (2014). CCR5 as a treatment target in pulmonary arterial hypertension. Circulation 130, 880–891. doi: 10.1161/CIRCULATIONAHA.114.010757
Annex, B. H. (2013). Therapeutic angiogenesis for critical limb ischaemia. Nat. Rev. Cardiol. 10, 387–396. doi: 10.1038/nrcardio.2013.70
Assenov, Y., Ramírez, F., Schelhorn, S. E., Lengauer, T., and Albrecht, M. (2008). Computing topological parameters of biological networks. Bioinformatics 24, 282–284. doi: 10.1093/bioinformatics/btm554
Azuma, K., Osaki, T., Kurozumi, S., Kiyose, M., Tsuka, T., Murahata, Y., et al. (2015). Anti-inflammatory effects of orally administered glucosamine oligomer in an experimental model of inflammatory bowel disease. Carbohydr. Polym. 115, 448–456. doi: 10.1016/j.carbpol.2014.09.012
Baños, M., Arellano-Mendoza, M. G., Vargas-Robles, H., Avila-Casado, M. C., Soto, V., Romo, E., et al. (2011). Relationship between angiotensin II receptor expression and cardiovascular risk factors in Mexican patients with coronary occlusive disease. Exp. Mol. Pathol. 91, 478–483. doi: 10.1016/j.yexmp.2011.05.002
Baslund, B., Wienecke, A. K., Rasmussen, N., Faurschou, M., and Toft, P. B. (2012). Treatment of orbital inflammation with rituximab in Wegener’s granulomatosis. Clin. Exp. Rheumatol. 30, 57–510.
Bech, J. J., Topol, E. J., Agnelli, G., Bertrand, M., Califf, R. M., Clement, D. L., et al. (2003). Critical issues in peripheral arterial disease detection and management: a call to action. Arch. Intern. Med. 163, 884–892. doi: 10.1001/archinte.163.8.888
Bilecen, D., Schulte, A. C., Kaspar, A., Küstermann, E., Seelig, J., Elverfeldt, D., et al. (2003). Detection of the non-steroidal anti-inflammatory drug niflumic acid in humans: a combined 19F-MRS in vivo and in vitro study. NMR Biomed. 16, 144–151. doi: 10.1002/nbm.820
Björck, M., Lepkowska Erikkson, M., Bylock, A., Steuer, J., Wanhainen, A., Carlsson, B. C., et al. (2013). Plasminogen activator inhibitor-1 levels and activity decrease after intervention in patients with critical limb ischaemia. Eur. J. Vasc. Endovasc. Surg. 46, 214–222. doi: 10.1016/j.ejvs.2013.05.011
Botti, C., Maione, C., Dogliotti, G., Russo, P., Signoriello, G., Molinari, A. M., et al. (2012). Circulating cytokines present in the serum of peripheral arterial disease patients induce endothelial dysfunction. J. Biol. Regul. Homeost. Agents 26, 67–79.
Buritova, J., and Besson, J. M. (1997). Potent anti-inflammatory/analgesic effects of lornoxicam in comparison to other nsaid:s a c-fos study in the rat. Inflammopharmacology 5, 331–341. doi: 10.1016/s1087-997-0300-9
Buritova, J., and Besson, J. M. (1998). Dose-related anti-inflammatory/analgesic effects of lornoxicam: a spinal c-Fos protein study in the rat. Inflamm. Res. 47, 18–25. doi: 10.1007/s000110050245
Cao, Z., Ding, X., Huang, Y., Liu, S., Lin, J., Lyu, H., et al. (2015). Etanercept inhibits synovial inflammation and reduces the expression of adhesion related molecules in synovial tissues of patients with rheumatoid arthritis. Xi Bao Yu Fen Zi Mi Xue. 31, 511–515.
Caracas, H. C., Maciel, J. V., Martins, P. M., de Souza, M. M., and Maia, L. C. (2009). The use of lidocaine as an anti-inflammatory substance: a systematic review. J. Dent. 37, 93–97. doi: 10.1016/j.jdent.2008.10.005
Chamian, F., Lowes, M. A., Lin, S. L., Lee, E., Kikuchi, T., Gillespaul, E., et al. (2005). Alefacept reduces infiltrating T cells, activated dendritic cells, and inflammatory genes in psoriasis vulgaris. Proc. Natl. Acad. Sci. U.S.A. 102, 2075–2080. doi: 10.1073/pnas.0409569102
Chen, Y. F., Jobanputra, P., Barton, P., Bryan, S., Fry-Smith, A., Harris, G., et al. (2008). Cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs (etodolac, meloxicam, rofecoxib, etoricoxib, valdecoxib and lumiracoxib) for osteoarthritis and rheumatoid arthritis: a systematic review and economic evaluation. Health Technol. Assess. 12, 1–278, iii. doi: 10.3310/hta12110
Chmiel, J. F., Konstan, M. W., Accurso, F. J., Lymp, J., Mayer-Hamblett, N., Vanderpenter, D. R., et al. (2015). Use of ibuprofen to assess inflammatory biomarkers in induced sputum: implications for clinical trials in cystic fibrosis. J. Cyst. Fibros. doi: 10.1016/j.jcf.2015.03.007. [Epub ahead of print].
Choi, E. K., Kim, S. H., Kang, I. C., Jeong, J. Y., Koh, J. T., Lee, B. N., et al. (2013). Ketoprofen inhibits expression of inflammatory mediators in human dental pulp cells. J. Endod. 39, 764–767. doi: 10.1016/j.joen.2013.02.003
Chou, W. Y., Chuang, K. H., Sun, D., Lee, Y. H., Kao, P. H., Lin, Y. Y., et al. (2015). Inhibition of PKC-induced COX-2 and IL-8 expression in human breast cancer cells by glucosamine. J. Cell. Physiol. 230, 2240–2251. doi: 10.1002/jcp.24955
Chu, L. H., Lee, E., Bader, J. S., and Popel, A. S. (2014). Angiogenesis interactome and time course microarray data reveal the distinct activation patterns in endothelial cells. PLoS ONE 9:e110871. doi: 10.1371/journal.pone.0110871
Fehervari, M., Krepuska, M., Szeplaki, G., Apor, A., Sotonyi, P., Prohaszka, Z., Francisci, D., Falcinelli, E., Baroncelli, S., Petito, E., Cecchini, E., Weimer, L. E., et al. (2014). Potential anti-inflammatory effects of maraviroc in HIV-positive patients: a pilot study of inflammation, endothelial dysfunction, and coagulation markers. *Scand. J. Infect. Dis.* 46, 466–470. doi:10.1080/03695548.2014.898332

Frostegärd, J. (2013). Immunity, atherosclerosis and cardiovascular disease. *BMC Med.* 11:117. doi:10.1186/1741-7015-11-117

Fruminkin, L. R. (2012). The pharmacological treatment of pulmonary arterial hypertension. *Pharmacol. Rev.* 64, 583–620. doi:10.1124/jr015.1105587

Fu, S., Zhao, H., Shi, J., Abghanov, A., Crawford, K., Ohno-Machado, L., et al. (2008). Peripheral arterial occlusive disease: global gene expression analyses suggest a major role for immune and inflammatory responses. *BMC Genomics* 9:369. doi:10.1186/1471-2164-9-369

Futosi, K., Németh, T., Pick, R., Vantus, T., Walzog, B., and Mócsai, A. (2012). Dasatinib inhibits proinflammatory functions of mature human neutrophils. *Blood* 119, 4981–4991. doi:10.1182/blood-2011-07-369041

Gardner, A. W., Parker, D. E., Montgomery, P. S., Sosnowska, D., Casanegra, A. L., Esporda, O. L., et al. (2014). Impaired vascular endothelial growth factor A and inflammation in patients with peripheral artery disease. *Angiology* 65, 683–690. doi:10.1177/0003317713510376

Glaser, K. B., Carlson, R. P., Sung, A., Bauer, J., Lock, Y. W., Holloway, D., et al. (1993). Pharmacological characterization of WAY-121,520: a potent anti-inflammatory indomethacin-based inhibitor of 5-lipoxygenase (5-LO)/phospholipase A2 (PLA2). *Agents Actions Spec No* C30–C32. doi:10.1007/BF01972711

Goertz, O., Ring, A., Buschhaus, B., Hirsch, T., Daigeler, A., Steinstraesser, L., et al. (2014). Influence of anti-inflammatory and vasoactive drugs on microcirculation and angiogenesis after burn in mice. *Burns* 37, 654–664. doi:10.1016/j.burns.2011.01.004

Goldfine, A. B., Silver, R., Aldlahi, W., Cai, D., Tatro, E., Lee, J., et al. (2008). Use of salbutamol to target inflammation in the treatment of insulin resistance and type 2 diabetes. *Clin. Transl. Sci.* 1, 36–43. doi:10.1111/j.1751-8423.2008.00026.x

Grisar, J. C., Haddad, F., Comari, F. A., and Wu, J. C. (2011). Endothelial progenitor cells in cardiovascular disease and chronic inflammation: from biomarker to therapeutic agent. *Biomark. Med.* 5, 731–744. doi:10.2217/bmm.11.92

Haas, T. L., Lloyd, P. G., Yang, H. T., and Terjung, R. L. (2017). Exercise training and peripheral arterial disease. *Compr. Physiol.* 2, 2933–3017. doi:10.1002/cphy.c110065

Hacke, W., Kaste, M., Bluhmki, E., Brotmann, M., Dávalos, A., Guidetti, D., et al. (2008). Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N. Engl. J. Med.* 359, 1317–1329. doi:10.1056/NEJMoa0804656

Hall, P. S., Hanby, A., and Cameron, D. A. (2009). Laptapin for inflammatory breast cancer. *Lancet Oncol.* 10, 538–539. doi:10.1016/S1470-4249(09)70120-2

Hazarika, S., Farber, G. R., Dokun, A. O., Pittalis, A., Wang, T., Lye, R. J., et al. (2013). MicroRNA-93 controls perfusion recovery after hindlimb ischemia by modulating expression of multiple genes in the cell cycle pathway. *Circulation* 127, 1818–1828. doi:10.1161/CIRCULATIONAHA.112.008660

Heilman, R. L., Khamash, H. A., Smith, M. L., Chakrera, H. A., Moss, A. A., and Reddy, K. S. (2013). Delayed allograft inflammation following alemtuzumab induction for kidney transplantation. *Clin. Transplant.* 27, 772–780. doi:10.1111/ctt.12201

Herová, M., Schmid, M., Gumperle, C., Loretz, C., and Hersberger, M. (2014). Low dose aspirin is associated with plasma chemerin levels and may reduce adipose tissue inflammation. *Atherosclerosis* 235, 256–262. doi:10.1016/j.atherosclerosis.2014.05.912

Hirano, K., Kemmotsu, Y., Wittkowski, H., Foell, D., Saito, K., Ikubi, K., et al. (2009). Infliximab reduces the cytokine-mediated inflammation but does not suppress cellular infiltration of the vessel wall in refractory Kawasaki disease. *Pediatr. Res.* 65, 696–701. doi:10.1038/PDR.08013e31819ed68d

Hirsch, A. T., Haskal, Z. J., Hertzler, N. R., Bakal, C. W., Creager, M. A., Halperin, J. L., et al. (2006). ACC/AHA Guidelines for the Management of Patients with Peripheral Arterial Disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (writing committee to develop guidelines for the management of patients with peripheral arterial disease)—summary of...
Rajpal, R. K., Ross, B., Rajpal, S. D., and Hoang, K. (2014). Bromfenac ophthalmic solution for the treatment of postoperative ocular pain and inflammation: safety, efficacy, and patient adherence. Patient Prefer. Adherence 8, 925–931. doi: 10.2147/PPA.S66667

Rice, T. W., and Bernard, G. R. (2004). Drotrecogin alfa (activated) for the treatment of severe sepsis and septic shock. Am. J. Med. Sci. 328, 205–214. doi: 10.1097/00000441-200401000-00003

Ridker, P. M., and Lüscher, T. F. (2014). Anti-inflammatory therapies in cardiovascular disease. Eur. Heart J. 35, 1782–1791. doi: 10.1093/eurheartj/ehu203

Ridker, P. M., Thuren, T., Zalewski, A., and Libby, P. (2011). Interleukin-1beta modifiers in systemic sclerosis. Crit. Rev. Oncol. Hematol. 77, 15, 2741–2749. doi: 10.1016/j.critrevonc.2010.09.001

Rull, A., García, R., Fernández-Sender, L., Beltrán-Debón, R., Aragonès, G., Alegría, J. M., et al. (2011). The role of combined assessment of defense molecule expression and phenotype at the single cell level for the identification of circulating tumor cells in non-small cell lung cancer patients. Cancer Lett. 306, 597–605. doi: 10.1016/j.canlet.2011.02.004

Sahara, M., Sata, M., Morita, T., Nakajima, T., Hirata, Y., and Nagai, R. (2010). Cytoskeleton remodeling during development of focal adhesions on angiogenic microvessels. Circ. J. 66, 385–391. doi: 10.1253/circj.CJ-09-0516

Shmilovich, H., Ben-Shoshan, J., Tal, R., Afek, A., Barschak, I., Maysel-Auslander, S., et al. (2009). B-type natriuretic peptide enhances vasculogenesis by promoting number and functional properties of early endothelial progenitor cells. Tissue Eng. Part A 15, 2741–2749. doi: 10.1089/teng.2008.0414

Signorelli, S. S., Fiore, V., and Malaponte, G. (2014). Inflammation and peripheral arterial disease: the value of circulating biomarkers (Review). Int. J. Mol. Med. 33, 777–783. doi: 10.3892/ijmm.2014.1657

Smoot, M. E., Ono, K., Ruscheinski, J., Wang, P. L., and Ideker, T. (2011). Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics 27, 431–432. doi: 10.1093/bioinformatics/btq675

Stati, T., Musumeci, M., Macciari, S., Massimi, A., Corritore, E., Strimpakos, G., et al. (2014). beta-Blockers promote angiogenesis in the mouse aortic ring assay. J. Cardiovasc. Pharmacol. 64, 21–27. doi: 10.1097/FJC.0000000000000887

Sun, J., Wu, Y., Xu, H., and Zhao, Z. (2012). D’Tome: a web-based tool for drug-target interaction construction. BMC Bioinformatics 13(Suppl. 9):S7. doi: 10.1186/1471-2105-13-S9-S7

Szubert, M., Suzin, J., Ducheler, M., Szuwalowska, A., Czyz, M., and Kowalczyk-Amico, K. (2014). Evaluation of selected angiogenic and inflammatory markers in endometriosis before and after danazol treatment. Reprod. Fertil. Dev. 26, 414–420. doi: 10.1071/RD12228

Taguchi, I., Toyoda, S., Takano, K., Arikawa, T., Kikuchi, M., Ogawa, M., et al. (2013). Iresistant, an angiotensin receptor blocker, exhibits metabolic, anti-inflammatory and antioxidative effects in patients with high-risk hypertension. Hypertens. Res. 36, 608–613. doi: 10.3109/hr.2013.3

Tang, E. H., Libby, P., Vanhoutte, P. M., and Xu, A. (2012). Anti-inflammation therapy by activation of prostaglandin EP4 receptor in cardiovascular and other inflammatory diseases. J. Cardiovasc. Pharmacol. 59, 116–123. doi: 10.1097/FJC.0b013e318244a412

Thomas, G. W., Rael, L. T., Shimonkevitz, R., Curtis, C. G., Bar-Or, R., and Bar-Or, D. (2007). Effects of danazol on endothelial cell function and angiogenesis. Fertil. Steril. 88, 1065–1070. doi: 10.1016/j.fertnstert.2006.11.179

Topol, E. J., Smith, J., Plow, E. F., and Wang, Q. K. (2006). Genetic susceptibility to myocardial infarction and coronary artery disease. Hum. Mol. Genet. 15 Spec No. 2, R117–R123. doi: 10.1093/hmg/ddl183

Varela, C., de Haro, J., Bleda, S., Rodríguez-Padilla, J., Ferrueto, A., and Acín, F. (2013). Circulating anti-beta2-glycoprotein 1 antibodies of peripheral arterial disease patients trigger a genomic overexpression of Toll-like receptor 4 in endothelial cells. J. Vasc. Surg. 61, 1041.e1–1041.e9. doi: 10.1016/j.jvs.2013.11.066

Varma, A., Das, A., Hoke, N. N., Durrant, D. E., Salloum, F. N., and Kukreja, R. C. (2012). Anti-inflammatory and cardioprotective effects of tadalafil in diabetic mice. PLoS ONE 7:e45243. doi: 10.1371/journal.pone.0045243

Wang, Y., Li, Y., Shen, Q., Li, X., Lu, J., Li, X., et al. (2014). Valsartan blocked alcohol-induced Toll-like receptor 2 signaling-mediated inflammation in human vascular endothelial cells. Alcohol. Clin. Exp. Res. 38, 2529–2540. doi: 10.1111/acr.12532

Weyrich, A. S., Denis, M. M., Kuhlmann-Eyre, J. R., Dixon, D. A., Marathe, G. K., et al. (2005). Dipyridamole selectively inhibits inflammatory gene expression in platelet-monocyte aggregates. Circulation 111, 633–642. doi: 10.1161/01.01.2005.085064.65

Whirl-Carrillo, M., McDonagh, E. M., Hebert, J. M., Gong, L., Sangkuhl, K., Thorn, C. F., et al. (2012). Pharmacogenomics knowledge for personalized medicine. Clin. Pharmacol. Ther. 92, 414–417. doi: 10.1038/clpt.2012.96

Willsiams, C., Panaccione, R., Ghosh, S., and Riaus, K. (2011). Optimizing clinical use of mesalazine (5-aminosalicylic acid) in inflammatory bowel disease. Therap. Adv. Gastroenterol. 4, 237–248. doi: 10.1177/1756283X11405250

Wozniak, K., Sleszycka, J., Safianowska, A., Wiechno, W., and Domagala-Kulawik, J. (2012). Systemic inflammation in peripheral arterial disease with or without coexistent chronic obstructive pulmonary disease: analysis of selected markers. Arch. Med. Sci. 8, 477–483. doi: 10.5114/ams.2012.29525

Writing Group, M., Lloyd-Jones, D., Adams, R. J., Brown, T. M., Carnethon, M., Dai, S., et al. (2010). Heart disease and stroke statistics–2010 update: a report from the American Heart Association. Circulation 121, e66–e215. doi: 10.1161/CIRCULATIONAHA.109.192667

Yan, S. N., Zhao, N. W., Zhu, X. X., Wang, Q., Wang, H. D., Fu, R., et al. (2013). Benzapreil inhibited the NF-kappaB and TGF-beta networking on LV hypertrophy in rats. Immunol. Lett. 152, 126–134. doi: 10.1016/j.imlet.2013.05.005

Yang, M., Cao, L., Xie, M., Yu, Y., Kang, R., Yang, L., et al. (2013). Chloroquine inhibits HMGB1 inflammatory signaling and protects mice from lethal sepsis. Biochem. Pharmacol. 86, 410–418. doi: 10.1016/j.bcp.2013.05.013

Yasar, A. S., Erbay, A. R., Ayaz, S., Turhan, H., Metin, F., Ilkay, E., et al. (2007). Increased platelet activity in patients with isolated coronary artery ectasia. Coron. Artery Dis. 18, 451–454. doi: 10.1097/MCA.0b013e3282a30665

Yu, C., Gong, R., Rifaï, A., Tolbert, E. M., and Dworkin, L. D. (2007). Long-term, high-dosage candesartan suppresses inflammation and injury in chronic kidney
disease: nonhemodynamic renal protection. J. Am. Soc. Nephrol. 18, 750–759. doi:10.1681/ASN.2006070770

Zachman, A. L., Wang, X., Tucker-Schwartz, J. M., Fitzpatrick, S. T., Lee, S. H., Guelcher, S. A., et al. (2014). Uncoupling angiogenesis and inflammation in peripheral artery disease with therapeutic peptide-loaded microgels. Biomaterials 35, 9635–9648. doi:10.1016/j.biomaterials.2014.08.011

Ziche, M., Morbidelli, L., Masini, E., Amerini, S., Granger, H. J., Maggi, C. A., et al. (1994). Nitric oxide mediates angiogenesis in vivo and endothelial cell growth and migration in vitro promoted by substance P. J. Clin. Invest. 94, 2036–2044. doi:10.1172/JCI117557

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2015 Chu, Annex and Popel. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.