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María D. Valls, Bruce N. Cronstein, M. Carmen Montesinos

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5 **Adenosine receptor agonists for promotion of dermal wound healing**  
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7 María D. Valls<sup>1</sup>, Bruce N. Cronstein<sup>2</sup>, M. Carmen Montesinos<sup>1,2</sup>  
8  
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10  
11  
12 **<sup>1</sup>Department de Farmacologia**  
13

14 **Universitat de València**  
15

16 **Ave. Vicent Andrés Estellès s/n**  
17

18 **46100 Burjassot, Valencia, Spain**  
19  
20  
21  
22  
23

24 **<sup>2</sup>Department of Medicine**  
25

26 **New York University School of Medicine**  
27

28 **550 First Ave.**  
29

30 **New York, NY 10016**  
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11  
12 **Corresponding author:**  
13

14 M. Carmen Montesinos,  
15

16  
17 Departament de Farmacologia / Universitat de València,  
18

19 Avenida Vicent Andrès Estellès s/n  
20

21 46100 Burjassot, Spain  
22

23  
24 Tel : +(34) 96 354 4946  
25

26 Fax : +(34) 96 354 4946  
27

28  
29 **[m.carmen.montesinos@uv.es](mailto:m.carmen.montesinos@uv.es)**  
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3 **ABSTRACT**  
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7 Wound healing is a dynamic and complex process that involves a well coordinated, highly  
8 regulated series of events including inflammation, tissue formation, revascularization and tissue  
9 remodeling. However, this orderly sequence is impaired in certain pathophysiological conditions  
10 such as diabetes mellitus, venous insufficiency, chronic glucocorticoid use, aging and  
11 malnutrition. Together with proper wound care, promotion of the healing process is the primary  
12 objective in the management of chronic poorly healing wounds. Recent studies have  
13 demonstrated that  $A_{2A}$  adenosine receptor agonists promote wound healing in normal and  
14 diabetic animals and one such agonist, Sonedenoson, is currently being evaluated as a prospective  
15 new therapy of diabetic foot ulcers. We will review the mechanisms by which adenosine receptor  
16 activation affects the function of the cells and tissues that participate in wound healing,  
17 emphasizing the potential beneficial impact of adenosine receptor agonists in diabetic impaired  
18 healing.  
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38 **Keywords:** Adenosine receptors; Impaired healing; Inflammation; Diabetic foot ulcer;  
39 angiogenesis; Granulation tissue  
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## Introduction

The mechanisms underlying the normal repair process, cell migration and proliferation, and extracellular matrix deposition and remodelling, have been extensively studied [1-3]. Cellular responses to inflammatory mediators, growth factors, cytokines, and to mechanical forces must be appropriate and precise in order to obtain optimum healing of a cutaneous wound. However, even during the normal process of wound healing complications can occur, including infection, thrombosis, and ischemia. [4, 5]. More importantly, the orderly progression of the healing process is impaired in chronic wounds, including those due to diabetes.

Impaired wound healing is a major concern for diabetic patients because their wounds do not heal properly and are a source of major suffering and cost. Only two-thirds of diabetic foot ulcers eventually heal and up to 28% may result in amputation [6]. The pathogenesis of diabetic foot ulcers is complex and it is well recognized that a number of contributory factors working together ultimately lead to impaired healing. Several intrinsic factors, such as peripheral neuropathy, foot deformity, peripheral vascular disease and peripheral oedema have been identified as the commonest factors responsible of impaired healing after trauma. In addition, extrinsic factors, such as wound infection, callus formation, and excessive pressure to the site, further aggravate the healing process [7].

Recent studies suggest that nerves play a central role in tissue homeostasis and can orchestrate complex reparative as well as destructive processes. First, an intact nociceptor system of primary afferent sensory nerves is important for the initiation of the inflammatory process and successful tissue repair [8]. Apart from the loss of pain perception, which is a key factor in the development

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3 of neuropathic foot ulcers, loss of autonomic function and small fibre neuropathy can result in  
4  
5 impaired neurogenic control of local microcirculatory blood flow, impaired fluid homeostasis,  
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7 diminished energy metabolism, oxygen delivery, and inflammatory responses. These processes  
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9 could render the feet of diabetic patients with neuropathy more susceptible to tissue damage and  
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11 infection [9].  
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16 Given the complexity of the pathogenesis of diabetic foot ulcers, many different interventions  
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18 have been proposed to accelerate the healing process, but few have been subjected to formal  
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20 evaluation. Despite the relatively large number of studies of growth factors like PDGF  
21  
22 (becaplermin), EGF, basic FGF and other agents modulating aspects of wound physiology, there  
23  
24 is currently little evidence to suggest that any of the reported interventions should be adopted in  
25  
26 routine practice. Diabetic foot ulcer management is based on the simple principles of eliminating  
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28 infection, debridement, cleansing and the use of dressings to maintain a moist wound bed, and  
29  
30 lastly, becaplermin is the only promoting agent approved for use of those ulcers resistant to  
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32 simpler interventions [6].  
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41 Based in studies carried out in vitro and in experimental animal models, we are proposing a new  
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43 strategy for the promotion of impaired wound healing, the use of adenosine receptor agonists.

44 We will summarized the biology of adenosine and review its actions on different tissues and cells  
45  
46 implicated in the healing of cutaneous wounds, as well as its effect on experimental wounds in  
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48 animals.  
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### Purine metabolism and biology

Adenosine is a ubiquitous purine nucleoside produced by stepwise dephosphorylation of ATP by the coordinated action of ecto-apyrase (CD39) and ecto-5'-nucleotidase (CD73) (Figure 1).

While extracellular ATP and other nucleotides (ADP, UTP and UDP) have many biological effects through direct activation of cell surface receptors for adenine nucleotides (seven P2X ionotropic and eight P2Y metabotropic receptor subtypes), adenosine modulates cellular and organ function via occupancy of four specific cell surface receptors, ( $A_1$ ,  $A_{2A}$ ,  $A_{2B}$  and  $A_3$ ), all members of the large family of 7-transmembrane spanning, heterotrimeric G protein-associated receptors [10, 11]. The  $A_1$  and  $A_3$  adenosine receptors coupled with  $G_i$  proteins are associated with two effector systems, namely, adenylate cyclase and phospholipase C. The binding of adenosine or its agonists to  $A_1$  and  $A_3$  adenosine receptors either induce inhibition of adenylate cyclase leading to a decrease in intracellular cAMP levels or stimulate phospholipase C and the release of intracellular  $Ca^{2+}$ .  $A_{2A}$  and  $A_{2B}$  receptors are associated with  $G_s$  proteins and their activation stimulates an increase in intracellular cAMP. In addition, they couple to mitogen-activated protein kinases (MAPK), which may give them a role in cell growth, survival, death and differentiation [12]. The affinity of selected agonists at the different adenosine receptor subtypes is summarized on Table 1.

Extracellular actions of purines in non-neuronal cells, including fast signalling roles in exocrine and endocrine secretion, platelet aggregation, cardiovascular effects and kidney function, have been known for a long time. More recently, slow purinergic signalling has been implicated in embryological development, wound healing, restenosis, atherosclerosis, ischaemia, cell turnover of epithelial cells in skin and visceral organs, inflammation, neuroprotection and cancer [13, 14].

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3 More interestingly, purines and pyrimidines have major roles in the activities of neurons. This  
4 includes nociceptive mechanosensory transduction, as well as acting as a cotransmitter and  
5 neuromodulator in most, if not all, nerve types in the peripheral and central nervous systems [13].  
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7 This raises the innovative hypothesis that diabetic patients suffering from peripheral neuropathy  
8 will have altered purinergic neurotransmission.  
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### 14 15 16 17 **Could adenosine play a role in normal wound healing?** 18

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20 Under basal conditions, the extracellular adenosine concentration is rather constant (30–300 nM),  
21 and held in tight check by the equilibrium between adenosine production/release into the  
22 extracellular space and adenosine uptake by cells or catabolism to inosine (Figure 1). In contrast  
23 its concentration can increase dramatically to micromolar or even higher ranges when there is an  
24 imbalance between energy use and energy supply, such as in oxygen depletion, or under  
25 conditions of cellular or tissue necrosis or stress as a result of ATP catabolism [12].  
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37 All cell subtypes involved in wound healing, macrophages, epidermal cells, fibroblasts and  
38 microvascular endothelial cells, differentially express functional adenosine receptors, although  
39 the receptor expression patterns vary between cellular types and have not been fully established.  
40 Moreover, even the same cellular type such as endothelial cells express different adenosine  
41 receptor subtypes depending on the vascular bed of origin [15]. Adenosine A<sub>2A</sub> receptors, in  
42 particular, are expressed on most cell types involved in wound healing, including macrophages,  
43 fibroblasts and microvascular endothelial cells [15-17]. We have reported that A<sub>2A</sub> receptor-  
44 deficient mice suffer from disordered wound healing with poor matrix formation and diminished  
45 blood vessel formation in the granulation tissue of excisional wounds and mechanically injured  
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3 skin, an observation that indicates a role for adenosine, acting at A<sub>2A</sub> receptors, in normal wound  
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5 healing [18]. We have further observed that cytokines released during the inflammatory phase of  
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7 wound healing, TNF- $\alpha$  and IL-1, up-regulate adenosine A<sub>2A</sub> receptor expression in human  
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9 monocytoid cells (THP-1) and microvascular endothelial cells [16, 19, 20]. Similarly, A<sub>2B</sub>  
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11 receptor expression in murine bone marrow-derived macrophages is also up-regulated by IFN-  
12  
13 gamma [21]. The differential adenosine receptor expression in cells from diabetic patients has not  
14  
15 been established yet. In this regard, streptozotocin-induced diabetes in rats altered adenosine  
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17 receptors expression in liver, heart and kidney and administration of insulin returned their levels  
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19 to normality [22]  
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### 26 **Adenosine in inflammation**

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31 Hemostasis and inflammation constitute the first phase of tissue repair. The formation of a blood  
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33 clot re-establishes tissue hemostasis and provides a provisional matrix for cell migration.  
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36 Released cellular mediators initiate inflammatory leukocyte recruitment necessary for removing  
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38 necrotic tissue and preventing infection [1]. In addition, inflammatory cells release a variety of  
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40 cytokines and chemokines that play an important role in the evolution of granulation tissue  
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42 through stimulation of fibroblasts and epithelial cells [4]. However, despite its importance,  
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44 persistent or exaggerated inflammation could be detrimental for the healing process in some  
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46 pathological settings. In this respect, the concept that diabetes is a low-level chronic  
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48 inflammatory disease is commonly accepted [23, 24].  
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3 Numerous studies indicate that adenosine, through the activation of its different receptor  
4 subtypes, is a potent regulator of inflammation and innate immunity [25, 26]. In fact, genetic  
5 deficiency in adenosine deaminase (ADA), the enzyme responsible of the deamination of  
6 adenosine to its less potent derivative inosine (Figure 1), is characterized by a severely  
7 compromised immune system [27]. Moreover, several studies have established that adenosine  
8 mediates the anti-inflammatory effect of mainstay anti-rheumatic drugs such as methotrexate and  
9 sulfasalazine, and also salicylates, in different animal models of acute and chronic inflammation  
10 [28-30].  
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24 Adenosine A<sub>1</sub> receptor activation has been associated with pro-inflammatory properties in most  
25 inflammatory cell types [31, 32]. Nevertheless, studies *in vivo* have demonstrated the anti-  
26 inflammatory effect of selective A<sub>1</sub> agonists acting in the Central Nervous System by increasing  
27 adenosine concentration at the inflamed site [33, 34].  
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36 Adenosine A<sub>2A</sub> receptors are generally regarded as the receptor subtype most relevant for the  
37 anti-inflammatory effect of adenosine. Their activation inhibits neutrophil and monocyte  
38 oxidative burst, degranulation and release of cytokines and chemokines [35, 36]. Activation of  
39 A<sub>2B</sub> receptors selectively inhibits collagenase mRNA accumulation in synovial fibroblasts,  
40 mediates neutrophil-stimulated intestinal epithelial leakiness and prevents vascular leakage and  
41 edema formation [37-39]  
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52 The role of adenosine A<sub>3</sub> receptors in inflammation has been more controversial, maybe due to  
53 the observed difference in agonist affinity between species. have also been described as anti-  
54 inflammatory in human blood leukocytes and in murine models of inflammation [40, 41]. We  
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3 have confirmed the anti-inflammatory effects of adenosine acting at A<sub>3</sub> receptors in experimental  
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5 animals, since animals deficient in this receptor show an exacerbated response to an  
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7 inflammatory insult when compared to their wild type littermates [42].  
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12 It has been firmly established that adenosine modulates the production of both inflammatory and  
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14 anti-inflammatory cytokines including TNF $\alpha$ , IL-10, and IL-12 [16, 43]. The anti-inflammatory  
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16 effect of adenosine could be beneficial since some reports depict a deleterious effect of TNF in  
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18 wound healing [44]  
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#### 24 **Adenosine in tissue formation**

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29 Driven by growth factors synthesized by local and migratory cells, fibroblasts migrate on the  
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31 provisional fibrin scaffold into the wound where they proliferate and construct a more robust  
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33 collagen rich extracellular matrix. Wound fibroblasts acquire a distinctive contractile and  
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35 secretory phenotype, known as myofibroblasts, responsible for wound contraction, a very  
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37 important event in full thickness wounds. In response to many of the same growth factors,  
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39 epidermal cells migrate from the edge of the wound over the surface of the injured area and  
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41 proliferate until there is complete wound closure [1, 5].  
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48 Many studies have shown that purinoceptors are involved in the regulation of proliferation and  
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50 differentiation of most target cells. Thus, activation of adenosine A<sub>2B</sub> receptor and P2Y<sub>2</sub>  
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52 receptors have mitogenic effects in murine keratinocytes [45], contrasting with earlier reports  
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54 showing that adenosine and its related nucleotides (ATP, ADP, AMP) were antiproliferative for  
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3 normal human epidermal keratinocytes cultured in the absence or presence of exogenous  
4 epidermal growth factor[46]. Similarly, the result of adenosine receptor activation in fibroblast  
5 proliferation remains unclear [47, 48]. Studies in our laboratory indicated that adenosine A<sub>2</sub>  
6 receptor occupancy, both A<sub>2A</sub> and A<sub>2B</sub>, contributes to enhanced fibroblast and endothelial cell  
7 migration.[49].  
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16 We have recently reported that activation of adenosine A<sub>2A</sub> receptors promotes collagen synthesis  
17 by human dermal fibroblasts and that blockade or deletion of this receptor in mice protects  
18 against bleomycin-induced dermal fibrosis, a murine model of scleroderma [17]. The stimulation  
19 of collagen synthesis in human dermal fibroblasts occurs through an A<sub>2A</sub>R/mitogen-activated  
20 protein kinase-1/mitogen-activated protein kinase-mediated pathway [17] Adenosine  
21 deaminase (ADA), the principal catabolic enzyme for adenosine in vivo, and its deficiency leads  
22 to the spontaneous development of pulmonary and skin fibrosis in mice, in which increased  
23 collagen deposition is accompanied by increased levels of key mediators of fibrosis, including  
24 transforming growth factor beta1, connective tissue growth factor, and interleukin-13.  
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38 Pharmacological treatment of ADA-deficient mice with the A<sub>2A</sub> receptor antagonist ZM-241385  
39 prevented the development of dermal fibrosis in this model of elevated tissue adenosine, by  
40 reducing dermal collagen content and expression of profibrotic cytokines and growth factors.  
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45 These data confirm a fibrogenic role for adenosine in the skin [50]. We also found an increased  
46 number of myofibroblasts associated with elevated skin adenosine concentration, a phenomenon  
47 that was prevented by pharmacological blockade of A<sub>2A</sub> receptors [51]. Although these results are  
48 consistent with a fibrogenic role for adenosine A<sub>2A</sub> receptor activation, a possible role for  
49 adenosine A<sub>2B</sub> receptors cannot be ruled out, since the concentration of the antagonist ZM-  
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3 241385 used in these studies is high enough to also antagonize rodent A<sub>2</sub>B receptors [52].  
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5 Wound healing models have not been studied in ADA- deficient mice.  
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### 8 9 **Adenosine in neovascularization**

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14 Revascularization of the wound bed is essential to supply oxygen, nutrients, and inflammatory  
15 cells to the newly growing tissue. Two mechanisms contribute to the development of new vessels  
16 in the adult: angiogenesis, the formation of new vessels from pre-existing ones; and  
17 vasculogenesis, the initial series of events in vascular growth in which endothelial cell precursors  
18 (angioblasts) differentiate in situ and assemble into solid endothelial cords [53] This multistep  
19 process is highly regulated by a variety of soluble angiogenic growth factors, proteolytic  
20 enzymes, which allow endothelial cell detachment and extracellular matrix invasion, and a close  
21 interaction between adhesive proteins of the extracellular matrix and their integrin receptors [54,  
22 55]. Among the growth factors implicated in the angiogenic process, bFGF and vascular  
23 endothelial growth factor (VEGF) are known to be potent angiogenic molecules that induce the  
24 growth of new blood vessels during wound healing and embryonic development. [56, 57].  
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40 Diabetic patients frequently suffer from macro and microvascular complications characterized by  
41 an early dysfunction of vascular endothelium that could further aggravate their impaired healing.  
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48 Many studies have shown that the administration of adenosine or adenosine agonists as well as  
49 the upregulation of endogenous adenosine can increase the expression of vascular endothelial  
50 growth factor (VEGF) in a variety of different cells studied in vitro [58-62] and after intravenous  
51 infusion of adenosine in humans [63]. Adenosine A<sub>2A</sub> receptors activation stimulates macrophage  
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3 production of VEGF as well [60], meanwhile adenosine A<sub>2B</sub> receptors induce VEGF release by  
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5 retinal endothelial cells [59, 61].  
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9 The elevated expression of VEGF might account for the angiogenic effects of adenosine;  
10  
11 however, it is more likely that adenosine also stimulates angiogenesis via other secondary  
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13 angiogenic and antiangiogenic mediators or by way of an intracellular action [64]. Thus, the  
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15 adenosine analog NECA also increased the expression of the proangiogenic factors insulin-like  
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17 growth factor-I (IGF-I) and basic fibroblast growth factor (bFGF) in human retinal endothelial  
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19 cells as well as the expression of the proangiogenic factors interleukin-8 (IL-8) and angiopoietin-  
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21 2 in human mast cells. A<sub>2B</sub> receptors mediated the IL-8 and bFGF responses and A<sub>3</sub> receptors  
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23 may have mediated the angiopoietin-2 response [65]. We have shown that adenosine and A<sub>2A</sub>  
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25 agonists can promote in vitro angiogenesis by inhibiting endothelial secretion of the anti-  
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27 angiogenic factor thrombospondin-1 [66].  
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### 36 **Adenosine in remodeling**

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41 The reparative process culminates with the remodelling of the newly formed granulation tissue in  
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43 order to restore complete functionality. During this extended phase, the provisional extracellular  
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45 matrix rich in type 3 collagen is degraded by serine proteases and metalloproteases and  
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47 sequentially substituted for the definitive matrix rich in type 1 collagen [1]  
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53 Activation of plasminogen plays a role in proteolytic degradation of extracellular matrices in  
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55 tissue remodelling events and it is required for normal repair of skin wounds in mice [67]. Earlier  
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57 reports showed that the non selective adenosine receptor agonist NECA increased plasminogen  
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3 activator release in rabbit alveolar macrophages due to intracellular elevations of cAMP [68].

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5 Similarly, cAMP elevating agents increased the acute release of tissue plasminogen activator in  
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7 human umbilical endothelial cells [69, 70] and intraarterial infusions of ATP, an adenosine  
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9 precursor, to healthy volunteers induce tPA release [71].  
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### 11 12 13 **Adenosine agonist promotion of wound healing in animal models**

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19 Much of the knowledge of the normal healing process of cutaneous wounds and the mediators  
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21 involved has evolved from information derived from experimental wounds in animals, especially  
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23 genetically modified mice. Unfortunately, a valid model of chronic wounds in animals has not yet  
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25 been developed. From the in vitro experiment data it is difficult to predict which adenosine  
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27 agonists, if any, will be useful for promoting impaired wound healing in humans.  
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34 There are few reports of the use of adenosine agonists for the enhancement of wound healing. We  
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36 have demonstrated pharmacologically and by the use of mice lacking  $A_{2A}$  receptors that topical  
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38 application of adenosine  $A_{2A}$  receptor agonists accelerate healing of dermal wounds in both  
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40 healthy animals and in streptozotocin-induced diabetic rats with impaired wound healing [18, 49,  
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42 72]. Histological analysis showed faster re-epithelialization and increased matrix deposition,  
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44 fibroblast density and vascularity in the granulation tissue of the agonist treated wounds as soon  
45  
46 as 3 days after injury [18]. In a model of pressure ulcer formation as a result of recurrent  
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48 ischemia-reperfusion of the skin, an adenosine  $A_{2A}$  receptor agonist infused via osmotic  
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50 minipumps reduced leukocyte infiltration and protected from ischemia/reperfusion injury in skin  
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53 [73]. No signs of fibrosis were described in these experimental animal models.  
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3 When studying the dose response effect of the selective A<sub>2A</sub> receptor agonist CGS-21680, a  
4 narrow therapeutic window was observed. Both low (0.5 µg /wound) and high concentrations (10  
5 µg /wound) did not affect the rate of wound closure; while intermediate doses were equally  
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10 effective. Loss of efficacy may be caused by the loss of specificity of CGS-21680 at higher  
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12 concentrations. In contrast Sonedenoson did not lose its accelerating effect at the highest  
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14 concentration studied. Moreover, it stimulated more rapid wound closure than CGS-21680,  
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16 suggesting the possibility that a higher selectivity of MRE0094 for A<sub>2A</sub> receptors may be  
17  
18 important [72]. It is worth noting that the pharmacokinetics of these compounds administered  
19  
20 topically in a hydrophilic gel have not been determined and could have important repercussions  
21  
22 in the final effect, in terms of penetration or systemic absorption and elimination rate. In the same  
23  
24 study 0.01% Becaplermin gel (rhPDGF-BB), the only growth factor approved for clinical use, did  
25  
26 not accelerate the rate of wound closure as compared to the control, and both CGS-21680 and  
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36 Topical application of the non selective adenosine receptor agonist 5'-N-ethyl-  
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38 carboxamidoadenosine (NECA) also normalized the impaired healing induced by subcutaneous  
39  
40 injection of dexamethasone- in mice. This effect was only partially abrogated by a selective A<sub>2B</sub>  
41  
42 antagonist, but the contribution of other receptors was not determined [45]. Topical application of  
43  
44 the adenosine A<sub>1</sub> receptor agonist, N(6)-Cyclopentyladenosine (CPA), also promoted healing of  
45  
46 incisional and excisional wounds on the dorsum of both diabetic (db/db) and normal (db/+) mice,  
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48 although the concentration used was above the selectivity threshold and the participation of other  
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5 Finally, using an incisional skin-wound model produced on the back of female diabetic mice,  
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7 intraperitoneal administration of Polydeoxyribonucleotide, a compound mixture of  
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9 deoxyribonucleotide polymers, improved the impaired wound healing and increased the wound-  
10  
11 breaking strength in diabetic mice through adenosine A<sub>2</sub> receptors activation. The promoting  
12  
13 effect of this compound was blocked by an antagonist, DMPX (3,7-dimethyl-1-  
14  
15 propargilxanthine), which blocks both A<sub>2A</sub> and A<sub>2B</sub> receptors [74].  
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22 As inferred from the in vitro studies previously described, many factors contribute to the effect of  
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24 adenosine receptor activation in promoting wound healing. The synergistic interaction between  
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26 Toll-like receptor and adenosine A<sub>2A</sub> receptor signaling switches macrophages towards an  
27  
28 angiogenic phenotype [60, 75], and plays a role in an excisional wound healing model. Mice  
29  
30 lacking MyD88, an adapter protein in the signal transduction pathway of Toll-like receptors, heal  
31  
32 at a markedly slower rate than wounds in wild-type mice, showing delayed contraction, decreased  
33  
34 and delayed granulation tissue formation, and reduced new blood vessel density. CGS21680, an  
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36 A<sub>2A</sub> receptor agonist, promoted wound closure and angiogenesis in wild type mice, but had no  
37  
38 significant effect on healing of MyD88(-/-) mice, suggesting the synergistic interaction between  
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40 Toll-like receptors and adenosine A<sub>2A</sub> receptors signaling in wound healing in vivo [76]  
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## 47 **Conclusion**

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52 Nowadays there is a high awareness of the problem that diabetic foot ulcers represent in terms of  
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54 costs and quality of life for those suffering them. Studies to better understand the normal healing  
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56 process and the pathology of impaired healing have been extremely useful to improve wound  
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3 management and care. Randomized controlled trials have shown that topical application of  
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5 becaplermin gel is effective in increasing healing rates for diabetic neuropathic foot ulcers with  
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7 adequate blood supply. However, this efficacy has not translated to positive clinical experience,  
8  
9 and the drug is not widely used. Moreover, it is an expensive medication of relative short storage  
10  
11 stability and an increased risk of mortality secondary to malignancy was observed in patients.  
12  
13 Adenosine receptor agonists represent an attractive and novel alternative to growth factors for  
14  
15 promotion of diabetic foot chronic ulcers. They are small synthetic molecules of longer stability  
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17 and lower cost than growth factors. Given the complexity of the pathogenesis of foot ulcers and  
18  
19 the differential adenosine receptor expression in the cells involved in the impaired tissue repair  
20  
21 process, it is difficult to predict which agonists, selective or not, could be most useful. To add  
22  
23 more complexity, all four receptor adenosine subtypes are G protein-coupled receptors, but also  
24  
25 exert cAMP-independent actions. In addition, they couple to mitogen-activated protein kinases by  
26  
27 mechanisms that appear to differ substantially, both between receptor subtypes in the same cell  
28  
29 type and between the same receptor in different cell types. From our studies, selective adenosine  
30  
31  $A_{2A}$  receptor agonists present the advantages of reducing inflammation and increasing  
32  
33 vascularization and extracellular matrix deposition. Nevertheless, the fibrogenic potential and  
34  
35 increased risk of infection should also be monitored. Other important factors to be considered  
36  
37 are the pharmaceutical formulation and the pharmacokinetics of the agonist used, in order to  
38  
39 minimize its systemic effects. As any new strategy in the management of chronic wounds, the use  
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41 of adenosine agonists has to be evaluated and validated before taking it into practice.  
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## 52 **Acknowledgments**

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3 **FIGURE LEGEND**  
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5 **Figure 1. Adenosine metabolism.** AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide;  
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7 AICART, 5-aminoimidazole-4-carboxamide ribonucleotide transformylase; FAICAR, formyl 5-  
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9 aminoimidazole-4-carboxamide ribonucleotide; ADA Adenosine deaminase; EctoADA  
10  
11 Ectoadenosineaminase; CD73 Ecto-5'-nucleotidase; CD39 ecto-nucleoside triphosphate  
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diphosphohydrolase (NTPDase); ENT1 equilibrative nucleoside transporter 1.

**Table 1. Binding affinity of selected adenosine receptor agonists at the four receptor subtypes**

<b>Ki (nM)</b>	<b>A<sub>1</sub></b>	<b>A<sub>2A</sub></b>	<b>A<sub>2B</sub></b>	<b>A<sub>3</sub></b>
Adenosine* [77]	310 (EC <sub>50</sub> )	700 (EC <sub>50</sub> )	24,000 (EC <sub>50</sub> )	290 (EC <sub>50</sub> )
2-Cl-adenosine [11]	1.39	180	N.D.	19
NECA[11]	14	20	330	6.2
CPA[11]	2.3	790	21,000	43
CGS21680[11]	290	27	361,000	67
ATL-146e [14]	77	0.5	N.D.	45
Sonedenoson (MRE0094)** [66, 72]	> 10,000 (IC <sub>50</sub> )	490 ± 50 (IC <sub>50</sub> )	> 10,000 (IC <sub>50</sub> )	N.D.
IB-MECA[77]	3.7	2,500	54,000	1.2

Ki, Dissociation constant of unlabeled compounds in radioligand competition experiments at recombinant human A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> adenosine receptors in Chinese hamster ovary (CHO) cells.

\* Adenosine data from a cyclic AMP functional assay in CHO cells stably transfected with recombinant human A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> adenosine receptors.

\*\* Data provided by King Pharmaceuticals at a variety of receptor systems by radioligand-binding studies.

N.D. Not determined or Not Disclosed

NECA 5'-N-ethyl-carboxamidoadenosine

CPA N6-cyclopentyladenosine

CGS 21680 2-[p-(2-carbonyl-ethyl)-phenylethylamino]-5'-N-ethylcarboxamidoadenosine

ATL146e 4-{3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl}-cyclohexanecarboxylic acid methyl ester

Sonedenoson (MRE0094) 2-[2-(4-chlorophenyl)ethoxy] adenosine

IB-MECA N6-(3-iodobenzyl)adenosine-5'-N-methyluronamide

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