Effects of diacerein at the molecular level in the osteoarthritis disease process

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***Abstract*:** In osteoarthritis (OA), the alterations in joint tissues are numerous and involve morphological, biochemical and metabolic changes and an upregulation of the inflammatory pathways. The focus of this article is a brief narrative review of the effects of diacerein, an antirheumatic drug from the anthraquinone chemical class, and its active metabolite, rhein, on the factors that participate in the complex interaction between OA tissues and cells leading to the progression of joint structural changes.

***Keywords*:** cartilage, diacerein, osteoarthritis, rhein, subchondral bone, synovial membrane

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Introduction

Osteoarthritis (OA) is a condition that represents a pathological imbalance in the degradative and reparative processes of the articular tissues. Although we still do not completely understand what initiates the degeneration of the articular tissues, significant progress has been made with respect to the pathogenesis of the disease. There is now evidence of a global cross-talk between the joint tissues, with the diffusion of catabolic factors from the synovial membrane and subchondral bone to the cartilage.

Although OA is characterized by a degeneration of articular cartilage, at the clinical stage of the disease this is accompanied by changes in the synovial membrane where an inflammatory reaction is often observed [Martel-Pelletier *et al.* 2005]. In addition, a complex relationship between the subchondral bone and the cartilage is currently regarded as a major pathophysiological factor in the progression of OA. Indeed, some continuity between subchondral bone and cartilage in OA has been demonstrated, which suggests a cross-talk between these tissues [Martel-Pelletier *et al.* 2007].

One hypothesis regarding the pathological development of OA at the clinical stage of the disease can be summarized as follows [Martel-Pelletier *et al.* 2005]. The cartilage matrix is first broken down by proteolytic enzymes. Matrix fragments are released into the fluid, which can promote inflammation in the synovial membrane. The inflammation of the membrane, through the synthesis of mediators, creates a vicious circle, in which the cartilage matrix is further degraded, subsequently provoking more inflammation. Several soluble mediators have been identified in articular tissues from arthritic diseases and studies have shown that inflammation in knee OA is primarily due to the presence of the cytokine interleukin-1β (IL-1β). Thus, IL-1β plays a fundamental role in the pathophysiology of OA, in which its catabolic effects are multiple: this cytokine is able to stimulate its own production, to increase the synthesis of catabolic factors as well as chondrocyte apoptosis, and to decrease some of the cartilage macromolecule synthesis. Therefore, targeting this cytokine and related factors is of great importance in therapeutic approaches to OA.

Diacerein/rhein

The current therapies for OA, including the non-steroidal anti-inflammatory drugs (NSAIDs), although effective against the disease symptoms, are palliative and do not stop the disease progression. There are, however, promising agents and compounds that have been shown to reduce the severity of the disease as well as the symptoms. Among them is diacerein, a drug belonging to the anthraquinone chemical class that is employed in the treatment of OA.

This article is a brief review of how its mechanism of action differs from that of a classic NSAID. Contrary to a classic NSAID that targets

Table 1. Summary of the effects of diacerein/rhein on articular joint tissues/cells.

Tissues/cells	Effects
Cartilage/chondrocytes and synovial membrane/synoviocytes	↓IL-1β system (IL-1β, ICE, IL-1RI)
	↓IL-1β-induced MMP-3, collagenase, ADAMTS-4, ADAMTS-5, NO, iNOS
	↑IL-1β-induced PGE_2, COX-2
	↓IL-1β-inhibition of collagen, proteoglycans
Subchondral bone	↓MMP-13
Osteoblasts	↓Vitamin D_3-induced osteocalcin
	↓uPA
	↑PGE_2/COX-2
Osteoclasts	↓MMP-13, cathepsin K
	↓Survival, differentiation

ADAMTS, disintegrin and metalloproteinase domain with thrombospondin motifs; COX-2, cyclooxygenase-2; ICE, IL-1 converting enzyme; IL-1β, interleukin-1β; IL-1RI, IL-1 receptor type I; iNOS, inducible nitric oxide synthase; MMP, metalloprotease; NO, nitric oxide; PGE_2, prostaglandin E_2; uPA, urokinase-type plasminogen activator.

cyclo-oxygenase (COX)-2, an enzyme responsible for prostaglandin production, diacerein is known to act on the IL-1β system.

Pharmacokinetics of diacerein/rhein

Diacerein in the body is entirely converted into rhein before reaching the systemic circulation. Rhein is either eliminated by the renal route or conjugated in the liver to rhein glucuronide and rhein sulfate. In turn, these metabolites are mainly eliminated by the kidneys [Nicolas *et al.* 1998]. Data also showed that the pharmacokinetics of diacerein are about the same in young healthy volunteers and elderly people, both after a single dose of 50 mg or twice daily for a total dose of 100 mg or 150 mg [Nicolas *et al.* 1998; Fedeli, 1988; Petitjean *et al.* 1991]. Pharmacokinetic studies of diacerein performed on healthy volunteers revealed that the plasma peak concentration of rhein after an oral administration was 10^{-5} M [Nicolas *et al.* 1998; Spencer and Wilde, 1997]. Moreover, a further study [Segré, 1988, reported in Sanchez *et al.* 2003], showed that following daily administration of oral diacerein at 50 mg every 12 hours for 1 month, rhein reaches the synovial fluid at concentrations of 10^{-6}–10^{-5} M.

In *in vitro* studies, the concentrations most used varied between 10^{-7} M and 10^{-4} M. The majority of the studies employed concentrations around 10^{-5} M, which is in the higher range reached in the synovial fluid. However, as treatment with diacerein is characterized by a slow onset of action, with a maximal clinical effect being reached after a few months (about 3 months), the concentrations utilized for *in vitro* studies thus mimic the effect observed *in vivo* attained after months of treatment.

Effects on cartilage and synovial membrane cells

IL-1β (Figure 1). Evaluation of the effects of diacerein and its active metabolite, rhein, on the production of IL-1β in human OA synovial membrane and cartilage showed that both drugs significantly decreased the synthesis of this cytokine (Table 1) [Martel-Pelletier *et al.* 1998].

IL-1β is synthesized in the cell as a biologically inactive precursor, which requires a proteolytic cleavage to permit the activation of the cytokine and the exiting of the cell. This is achieved through a highly selective protease, the IL-1-converting enzyme (ICE), also named caspase-1. Hence, the action of ICE on IL-1β appears to be a key limiting factor for the secretion and activity of this cytokine. ICE has been shown to be expressed and synthesized by both human synovial membrane and cartilage and its levels are elevated during the OA process [Saha *et al.* 1999]. Diacerein and rhein markedly and significantly decreased ICE production in cartilage [Moldovan *et al.* 2000].

The biological activation of cells by cytokines is mediated through an association with specific cell surface receptors. For IL-1β, this occurs through binding to two types of specific membrane receptors, types I and II; type I was shown to be responsible for mediating the signal. The levels

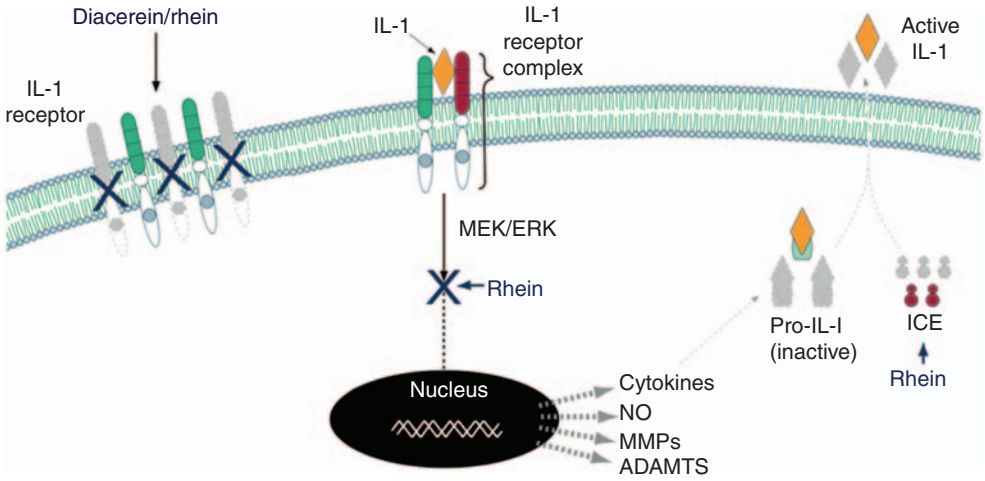

Figure 1. Effect of diacerein/rhein on the IL-1 system. Diacerein/rhein reduces the level of IL-1 receptors leading to fewer receptors to form heterodimer complexes. Following the association of IL-1 with its specific cell surface receptor complex, there is an activation of downstream signalling pathways involving some MAP kinases. In OA articular tissues, rhein has been shown to reduce numerous genes including cytokines, nitric oxide (NO), metalloproteases (MMPs), a disintegrin and metalloproteinase domain with thrombospondin motifs (ADAMTS), etc., through the inhibition of the MEK/ERK intracellular cascade. IL-1β is produced as a precursor which is cleaved at the cell membrane by the IL-1 converting enzyme (ICE) which releases IL-1β as an active cytokine into the extracellular matrix. Rhein reduces the production of ICE leading to a reduction in IL-1β activation. The grey color indicates that lesser amounts of the factors are produced. The figure is from and reproduced with permission of TRB Chemedica International. MEK = mitogen-activated protein kinase (MAP kinase), ERK = extracellular-signal-regulated kinase.

of this receptor type were also found to be markedly increased in OA chondrocytes and synovial fibroblasts, thus potentializing the effect of IL-1β activity [Sadouk *et al.* 1995; Martel-Pelletier *et al.* 1992; McCollum *et al.* 1991]. Investigation of the effects of diacerein and rhein on the binding and receptor levels in human OA chondrocytes showed that, at therapeutic concentrations, the drugs significantly inhibited the IL-1 binding level. Analysis of the competitive binding experiments revealed that both treated and untreated OA chondrocytes had similar IL-1 binding affinities, but that the receptor density, or number of receptors, was significantly reduced by both drugs [Martel-Pelletier *et al.* 1998].

Degradative enzymes. Further investigations were then directed to major catabolic pathways induced by IL-1β involved in the OA pathological process. In cartilage, the enzymatic matrix breakdown is a key feature in the progression of the disease. The loosening of the collagen network as well as the alterations in the aggrecan (proteoglycan) result from an increase in the amount of enzymes belonging to the MMP (metalloproteases) and ADAMTS (disintegrin and metalloproteinase domain with thrombospondin motifs) families. In regard to collagen degradation, three collagenases, MMP-1, MMP-8, and MMP-13, have been identified in humans, with high production levels found in OA. MMP-1 and MMP-13 are the major enzymes that account for collagen type II degradation in pathological cartilage. Moreover, it has been shown that in OA MMP-13 is produced during the remodelling phase, not only in the cartilage but also in the subchondral bone. Stromelysin-1 or MMP-3 is also considered an important enzyme in cartilage matrix turnover as, in addition to cleaving the proteoglycans, it is implicated in the enzymatic cascade responsible for the activation of proMMP-1. With regard to the proteoglycans, also found in OA articular tissues are aggrecan fragments with a proteolytic cleavage at the Glu373-Ala374 bond of the interglobular domain, between the G1 and G2 domains. The enzymes responsible for such cleavage belong to a subgroup of the ADAM family, the ADAMTS, and are named aggrecanases. Two such enzymes have been reported to be present in cartilage, ADAMTS-4 and ADAMTS-5. Recent studies in mice demonstrated that of the two aggrecanases, ADAMTS-5 is the predominant one involved in the OA degradative process [Stanton *et al.* 2005; Glasson *et al.* 2005]; however, in humans this still needs to be confirmed.

Data showed that diacerein and rhein significantly inhibited the IL-1β-stimulated MMP-3 and collagenase activity [Alvarez-Soria *et al.* 2008; Legendre *et al.* 2007; Sanchez *et al.* 2003; Tamura and Ohmori, 2001; Martel-Pelletier *et al.* 1998]. On the ADAMTS, both drugs decreased the IL-1β-stimulated ADAMTS-4 and ADAMTS-5 and a marked inhibition was found with rhein [Legendre *et al.* 2007].

Nitric oxide. Nitric oxide (NO) is produced through the activity of inducible nitric oxide synthase (iNOS) and is a major catabolic factor involved in the pathophysiology of OA. IL-1β is a very potent stimulator of NO. In OA, NO is involved in the promotion of cartilage catabolism and reduction in anabolism via a number of mechanisms. In brief, this factor reduces cartilage macromolecular synthesis and increases MMP activity, COX-2/prostaglandin E_2 (PGE_2) production, and apoptosis. Both diacerein and rhein treatments markedly and significantly decreased IL-1β-induced NO production [Sanchez *et al.* 2003; Pelletier *et al.* 1998]. Interestingly, in one experiment [Pelletier *et al.* 1998], in which an NSAID was used as comparator, data revealed that only a slight inhibition was obtained for the NSAID, and that occurred at a high concentration. This finding, among others, illustrates that these two classes of drugs have different mechanisms of action. Additional experiments also showed that diacerein and rhein reduced both the expression and production levels of iNOS [Pelletier *et al.* 1998].

Prostaglandin E_2 and cyclooxygenase-2. The effect of diacerein was also investigated on PGE_2 and on COX, as the latter is involved in one of the key steps in the synthesis of this prostanoid. As is well known, the spontaneous synthesis of PGE_2 or COX-2 in chondrocytes is low and their production is markedly increased following IL-1β treatment. In contrast to the effects of NSAIDs that reduce PGE_2 and COX-2, rhein and diacerein upregulate PGE_2 and COX-2 production [Sanchez *et al.* 2003; Pelletier *et al.* 1998]. These data correlate with other studies done with diacerein on other cell types [Alvarez-Soria *et al.* 2008; Pomarelli *et al.* 1980]. Although such elevation could appear detrimental in the context of OA, it is of interest that a metabolite of COX-2, 15-deoxy PGJ_2 (15d-PGJ_2), displays anti-inflammatory properties. 15d-PGJ_2 is a ligand of a nuclear receptor that exerts its effects possibly through binding to the peroxisome proliferator-activated receptor γ (PPARγ). The PPARs are a family of ligand-activated transcription factors, which, following ligand binding, heterodimerize with the retinoic X receptor (RXR) [Fahmi *et al.* 2002a]. This complex binds to PPAR-responsive elements (PPREs) in the promoter regions of target genes, thus inducing anti-inflammatory effects. Therefore, increasing COX-2 levels could lead to the formation of this metabolite, which would activate the PPARγ and abrogate the IL-1β-induced production of catabolic factors. Treatment of human chondrocytes with 15d-PGJ_2 resulted in the inhibition of IL-1β-induced NO and MMP-13 as well as proteoglycan degradation [Fahmi *et al.* 2001, 2002a, 2002b; Bordji *et al.* 2000]. Moreover, this PPARγ ligand also completely inhibited the effects of two other pro-inflammatory cytokines, tumor necrosis factor (TNF)-α and IL-17, on these cells. Similarly, PPARγ activators suppressed IL-1β-induced MMP-1 expression and production in human OA synovial fibroblasts [Fahmi *et al.* 2002c] and IL-1β and TNF-α expression in rheumatoid synovial fibroblasts [Ji *et al.* 2001]. In rat synovial fibroblasts, which also express PPARγ, 15d-PGJ_2 dose-dependently prevented lipopolysaccharide (LPS)-induced iNOS, COX-2, IL-1 and TNF-α expression [Simonin *et al.* 2002]. The protective effect of PPARγ activators has also been demonstrated in several animal models of arthritis including a guinea pig model of OA [Kobayashi *et al.* 2005]. Data showed that diacerein and rhein increased the activation of PPARγ (authors' personal data). Thus, the effect of increasing COX-2 might not be as damaging as it might seem, as the increased level could lead to the formation of a metabolite that would, in turn, activate a nuclear receptor with anticatabolic effects.

Apoptosis. The role of chondrocyte death by apoptosis in cartilage degradation is likely an important local factor contributing to the loss of matrix, and it has been reported that apoptosis is implicated in the loss of chondrocytes in OA [Blanco *et al.* 1998]. The involvement of the caspase cascade in cell death by apoptosis is well documented, and the caspases-3, -8, and -9 are the primary enzymes involved in cell apoptosis. These enzymes induce cell death by a number of mechanisms, including DNA fragmentation and inactivation of the proteins that protect cells against apoptosis. Data showed that rhein at a physiological concentration did not affect caspases-3/7, but at a concentration of about

10 times the physiological concentration, it induced a marked decrease in the activity of these enzymes in both synovial fibroblasts and chondrocytes [Legendre *et al.* 2009]. However, these authors also showed that DNA fragmentation was not induced by rhein at any of the concentrations used.

Cartilage matrix macromolecules. Data showed that diacerein and rhein decreased the inhibitory effect of IL-1 on the synthesis of collagen and proteoglycans [Domagala *et al.* 2006; Sanchez *et al.* 2003; Pujol *et al.* 2000; Yaron *et al.* 1999]. It was further suggested that this may occur through the stimulation of transforming growth factor (TGF)-β1 expression [Felisaz *et al.* 1999], as diacerein counteracts the IL-1β downregulation of matrix synthesis [Redini *et al.* 1988].

Effects on subchondral bone

Investigations were also performed on the effects of diacerein on subchondral bone. It is currently suggested that alterations in the subchondral bone may be more intimately related to the OA process and are not merely a consequence of the disease. Indeed, although cartilage degradation characterizes OA, there is evidence that the remodelling of subchondral bone is a contributing factor. Interestingly, although it was originally thought that the calcified cartilage layer was an impenetrable structure, in OA the presence of channels and microcracks between the subchondral region and the uncalcified cartilage has been demonstrated as well as vascularization in the subchondral bone [Sokoloff, 1993], which could favour the diffusion of factors from the subchondral bone region to the basal layer of cartilage and be responsible for the cartilage remodelling in the deep zone. In addition, recent work indicates that biological and morphological disturbances occur in this tissue very early on in the OA process, which may contribute to the initial events of the pathological process. However, human OA subchondral bone osteoblasts demonstrate an altered metabolic activity or phenotype compared to normal, in which elevated levels of the bone markers alkaline phosphatase and osteocalcin, and enzymes including the urokinase/plasmin system and MMP-13, for example, are found [Massicotte *et al.* 2002; Hilal *et al.* 1998, 1999]. Moreover, data also demonstrated that human OA subchondral bone, although showing sclerosis at a later stage, undergoes phases of bone resorption [Kwan Tat *et al.* 2008], and emerging data indicate a generalized undermineralization of OA subchondral bone [Couchourel *et al.* 2009]. Thus, therapeutic strategies aimed at modifying the metabolism of subchondral bone may be indicated in the treatment of OA.

Evaluation of the effects of diacerein and rhein on OA subchondral bone (Table 1) osteoblasts revealed that on the cell biomarkers, these drugs dose-dependently inhibited vitamin D_3-induced osteocalcin release [Pelletier *et al.* 2001]. This is interesting, as abnormally elevated osteocalcin levels have been observed in the subchondral bone of OA patients, and osteocalcin is believed to be involved in the local modulation of bone formation. Specifically, osteocalcin retards bone formation/mineralization. As OA subchondral bone is undermineralized, reducing osteocalcin would favour a more mineralized tissue. Of the metabolic factors, the production of urokinase-type plasminogen activator (uPA) by osteoblasts is inhibited by these drugs [Pelletier *et al.* 2001]. This reduction in uPA activity could retard bone formation by preventing the release of trapped growth factors, thus preventing further sclerosis. Moreover, the production of MMP-13 in this tissue is also inhibited by diacerein and rhein [Boileau *et al.* 2008]. This is important as MMP-13 acts directly to resorb bone; therefore, reducing the MMP-13 level would contribute to curbing bone resorption.

In bone biology, osteoblasts and osteoclasts contribute either alone or in combination to the remodelling process. The disturbance between the activities of these two cells is suggested to be responsible for the development of an altered bone metabolism. Investigation of diacerein and rhein on some parameters of the osteoclasts revealed that both drugs reduced not only MMP-13 but also cathepsin K [Boileau *et al.* 2008]. These data are important because, in osteoclasts, MMP-13 is known to work in conjunction with cathepsin K in the induction of bone resorption; therefore, the reduction in activity of these two enzymes would impact the balance between bone resorption and formation. Exploration of the effect of these drugs on osteoclast survival and differentiation showed that they effectively block not only the survival of mature osteoclasts but also the differentiation and proliferation of pre-osteoclasts into mature osteoclasts, the final effect being a reduction in the number of osteoclasts. Although further studies are needed to fully elucidate the precise mechanism of action of diacerein and rhein on osteoclasts, it may be

related to their effect on PGE_2, the levels of which, as mentioned above, are increased by these drugs in many cell types including human subchondral bone osteoblasts [Pelletier *et al.* 2001]. Hence, it has been reported that high PGE_2 levels inhibited bone resorption and that human OA subchondral bone osteoblasts expressing low levels of PGE_2 enhanced the formation of osteoclasts, whereas those expressing higher levels did not [Kwan Tat *et al.* 2008].

Effects on signalling pathways

The intracellular mechanisms by which these drugs exert their effect appear to occur through the down-regulation of the activation of some MAP kinases. Although other signalling pathways have been found for the different cells including the activation of c-Jun N-terminal kinase (JNK) on chondrocytes [Legendre *et al.* 2007; Martin *et al.* 2003] and p38 on osteoblasts [Boileau *et al.* 2008], it appears that on all articular cells, rhein reduces the catabolic pathways of OA through inhibition of MEK/ERK signalling [Boileau *et al.* 2008; Legendre *et al.* 2007; Domagala *et al.* 2006; Martin *et al.* 2003].

In vivo effects on the OA process in animal models and human clinical studies

The effect of diacerein was also studied *in vivo* in OA animal models, and data from studies on different animal models concur with those obtained *in vitro* with human articular cells. Indeed, in OA animal models, the drug has been shown to decrease the disease severity as well as the collagenase levels in the cartilage of dogs and rabbits [Brandt, 2006; Smith *et al.* 1999; Brandt *et al.* 1997; Mazieres *et al.* 1993, 1996], IL-1β and the loss of hydroxyproline and proteoglycans in mouse cartilage [Colville-Nash, 2002; Moore *et al.* 1997, 1998], iNOS and apoptosis in dog cartilage [Pelletier *et al.* 2003], and subchondral bone remodelling in sheep and rats [Tamura *et al.* 2002; Hwa *et al.* 2001; Ghosh *et al.* 1998].

Moreover, the conclusion of a meta-analysis [Rintelen *et al.* 2006] and data from a Cochrane review [Fidelix *et al.* 2006] indicate that oral diacerein demonstrated a good safety profile, was associated with significant improvement in symptoms of patients with hip and knee OA, had similar efficacy to NSAIDs but with a carry-over effect once treatment was stopped, and reduced NSAID consumption. In addition, an *in vivo* study in humans with hip OA showed that this drug demonstrates a structure-modifying effect [Dougados *et al.* 2001].

Conclusion

The data from basic research both *in vitro* and *in vivo* in animals provide evidence that diacerein treatment could impact the abnormal metabolism of OA articular tissues and cells by reducing the major catabolic processes, with a coherent body of evidence of its effect in clinical studies. Importantly, although an *in vivo* structure-modifying effect has been shown in humans with hip OA [Dougados *et al.* 2001], studies on the tissue structure need to be done in knee OA.

Even though such studies in knee OA have been impaired due to the imaging tools (X-ray) being unsatisfactory and having significant limitations, in recent years important advances have been made in the quantitative assessment of global structural changes in knee OA with the use of magnetic resonance imaging (MRI) to assess cartilage volume and thickness as well as synovial membrane and subchondral bone lesions [Raynauld *et al.* 2003, 2004, 2006, 2008a, 2008b; Pelletier *et al.* 2007, 2008; Berthiaume *et al.* 2005]. Such technology allows the analysis of OA disease progression over time and reduces the number of patients needed in clinical trials, improves retention of these patients, and reduces the overall costs and the length of clinical trials.

In conclusion, the current pharmacological management of OA is based primarily on the use of analgesics, NSAIDs, and antiCOX-2s. Although studies have confirmed the efficacy of NSAIDs and antiCOX-2s as symptomatic treatments for OA, these drugs have not proven to positively affect the natural course of OA in humans. The development of pharmacological agents capable of modifying the OA disease process is now crucial. In this context, basic research has shown that diacerein is an attractive candidate.

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Conflict of interest statement

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speakers for these two companies at international and local congresses and meetings.

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