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The Relationship between Chondroprotective and Antiinflammatory Effects of *Withania somnifera* Root and Glucosamine Sulphate on Human Osteoarthritic Cartilage *In Vitro*

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Using a validated explant model of *in vitro* cartilage damage, the effects of aqueous extracts of *Withania somnifera* (Ashwagandha) root and glucosamine sulphate (GlcS) were tested on the levels of nitric oxide (NO) and glycosaminoglycans (GAGs) secreted by knee cartilage from chronic osteoarthritis (OA) patients. *W. somnifera* extracts significantly decreased NO release by explants from one subset of patients (antiinflammatory response) and significantly increased levels of NO and GAGs released by explants from the second subset ('non-responders'). This is the first study showing direct, statistically significant, antiinflammatory effects of *W. somnifera* on human OA cartilage. It also confirmed that glucosamine sulphate exhibited statistically significant, antiinflammatory and chondroprotective activities in human OA cartilage. However, these beneficial effects of GlcS were observed in cartilage explants from 50% of patients tested ('responders'). In contrast, glucosamine significantly increased secretion of NO but not GAGs in explants from the second subset of OA patients ('non-responders'). Cartilage explants from the 11 OA patients gave differential responses to both drugs. Patient samples which responded to the antiinflammatory effects of *W. somnifera* did not always give a similar response to glucosamine, and vice versa. Thus, this *in vitro* model of human cartilage damage provides qualitative and statistically significant, quantitative pre-clinical data on antiinflammatory and chondroprotective activities of antiarthritic drugs. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: cartilage explants; *Withania somnifera*; chondroprotective; antiinflammatory.

INTRODUCTION

Osteoarthritis (OA) is a serious, degenerative disease affecting millions globally. Nonsteroid antiinflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs), immunosuppressants, biological response modifiers and genetically engineered drugs are being used to reduce pain and inflammation (Fajardo and Di Cesare, 2005). Slow-acting drugs such as chondroitin and glucosamine sulphate have shown promise. However, controversies on the therapeutic efficacy of glucosamine in OA prevail (da Camara and Dowless, 1998; Cibere *et al.*, 2004). Therefore, identification of new chondroprotective and antiinflammatory nutraceuticals assumes importance.

Root extracts of *Withania somnifera* (Ashwagandha), exhibit antioxidant, immunomodulatory and antiarthritic properties (Mishra *et al.*, 2000; Bani *et al.*, 2006). *Phyllanthus emblica* fruits (Amalaki) reportedly have rejuvenating and antiinflammatory properties in a rat model of joint disease (Ganju *et al.*, 2003). It was shown that both herbal extracts moderately inhibited collagenase type 2 activity, while *P. emblica* fruit extract also exhibited potent hyaluronidase inhibitory activity.

Studies on chondroprotective drugs report that proteoglycan (PG) release by cartilage explants is a proven marker of cartilage matrix damage *in vitro* (Nethery *et al.*, 1992). Therefore, the effects of these herbal drugs on the release of glycosaminoglycans (GAGs) by explant cultures of cartilage derived from OA patients were measured. With glucosamine sulphate as a positive control, the aqueous extracts of *W. somnifera* root and *P. emblica* fruit caused a statistically significant inhibition of GAGs released by cartilage explants from subsets of OA patients. These were the first reports demonstrating the chondroprotective activities of these Ayurvedic herbs on OA cartilage (Sumantran *et al.*, 2007a and b).

Nitric oxide (NO) plays a key regulatory role in pain, inflammation and the integrity of joint cartilage. Explant cultures of OA cartilage spontaneously release high levels of NO due to the activation of the inducible nitric oxide sythase (iNOS) (Pelletier *et al.*, 1996). Further, NO is known to mediate the inhibitory effects of the pro-inflammatory cytokine IL-1 beta, on the

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synthesis of cartilage matrix (Cai *et al.*, 2002). Thus, inhibitors of iNOs and NO release, such as tetracyclines, have a therapeutic role (Amin *et al.*, 1996).

This study presents new data on the effects of aqueous extracts of *W. somnifera* roots and glucosamine sulphate (GlcS) on cartilage damage. Cartilage damage was measured by assaying the levels of nitric oxide (NO) and glycosaminoglycans (GAGs) released by explants of OA cartilage taken from 11 patients prior to knee replacement surgery. Upon statistical analysis of the data, the existence of responders and non-responders to the antiinflammatory effects of *W. somnifera*, and to the chondroprotective and antiinflammatory effects of glucosamine sulphate were observed.

This is the first report demonstrating direct, statistically significant, antiinflammatory effects of *W. somnifera* on human OA cartilage. This report also provides an *in vitro* correlate for the clinical phenomenon of a 'partial drug response', i.e. the existence of responders and non-responders to the antiarthritic effects of glucosamine sulphate and *W. somnifera* on human OA cartilage *in vitro*. The physiological relevance of these data is discussed.

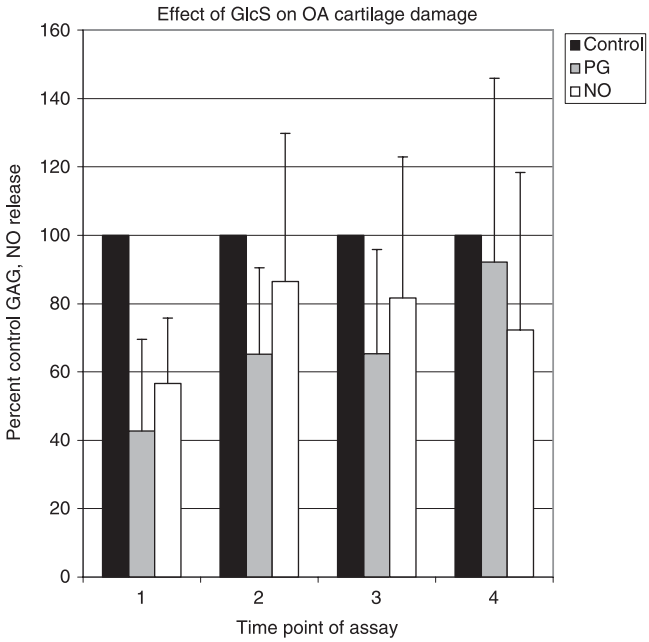

Figure 1. Chondroprotective and antiinflammatory effects of glucosamine on OA cartilage. Glucosamine sulphate extracts caused a statistically significant, short-term, decrease in GAGs (black versus grey bars) released by cartilage explants from five patients (OA 2, 4, 6, 8, 11) at time points 1 and 2. Glucosamine also induced a significant decrease in levels of NO released by these explants at time point 1 (black versus white bars). Levels of GAG and NO released by control explants from all patients are normalized to 100% (black bars).

MATERIALS AND METHODS

Materials. Roots of *W. somnifera* (crude drug sample No. ERH/47) were collected and authenticated at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. This herbal root powder was provided by the Council for Scientific and Industrial Research, (CSIR), India. The powder was extracted with hot water and spray dried. Standardization of aqueous extracts *W. somnifera* was done by HPLC (high performance liquid chromatography) using withaferin-A and withanolide-A as reference standards.

Preparation of aqueous extracts of *W. somnifera* root and glucosamine sulphate. Glucosamine sulphate or *W. somnifera* root powder were solubilized in distilled water (10 mg/mL) by limited autoclaving (5 pounds pressure for 7 min). Aqueous extracts of each drug were filter sterilized (0.45 μm CN membrane) in culture media before addition to the cells. Aqueous extracts of each drug were freshly prepared for each experiment.

This extraction method yielded drug extracts with reproducible sterility and bioactivity.

Osteoarthritis patients. Patients (55–75 years) suffered from chronic OA for 5–15 years prior to knee replacement surgery. The scale to assess cartilage integrity was as follows (Outerbridge, 1961). Grade 1: mild softening or blistering of articular cartilage.

Grade 2: Fragments/fissures in <1 cm^2 of the affected condylar cartilage.

Only non-calcified, articular cartilage (grades 1 or 2) from the lateral femoral condyles was used in this study.

Explant cultures of cartilage. The study tried to determine whether there were short- or long-term chondroprotective effects of the two drugs (*W. somnifera* root extract and glucosamine) on human OA cartilage. Therefore, cartilage damage was assayed over an 8 day period after a 24 h exposure to sterile, aqueous extracts of each drug. The detailed experimental design was as follows.

Explant cultures of OA knee cartilage (20–40 mg each) were set up in 24-well tissue culture plates. The growth media was 1:1 mixture of DMEM: Ham's F12 basal media with 10% heat inactivated fetal bovine serum + gentamicin (8 μg/mL). After 1 day in culture, cartilage explants were treated for 24 h with/without sterile aqueous extracts of either drug (0.05 mg/mL) in growth media. Next, each explant was refed with growth media without drug every 2 days for 8 days. Thus, conditioned media (CM) samples at four time points were available from each explant per patient (days 2, 4, 6 and 8, following the 24 h treatment of explants with/without drug). Figures 1 and 2 show time points labeled as 1–4, which correspond to CM samples collected on day 2, 4, 6 and 8 respectively, post treatment with drug. CM samples from each explant were collected prior to each re-feeding, and stored at –20 °C.

Assays for cartilage explant damage: glycosaminoglycans (GAG) and nitric oxide (NO). Total GAG levels in CM samples were measured by dimethylmethylene blue binding, using chondroitin sulphate as a standard (Hoemann *et al.*, 2002). NO levels in CM samples were estimated using Griess reagent. CM samples of cartilage explants from each OA patient were thawed only once, and assayed for glycosaminoglycans (GAG) and nitric oxide (NO) levels simultaneously. This precaution minimized variance.

Data analysis and presentation. Raw data for levels of NO and GAG in CM samples from controls versus

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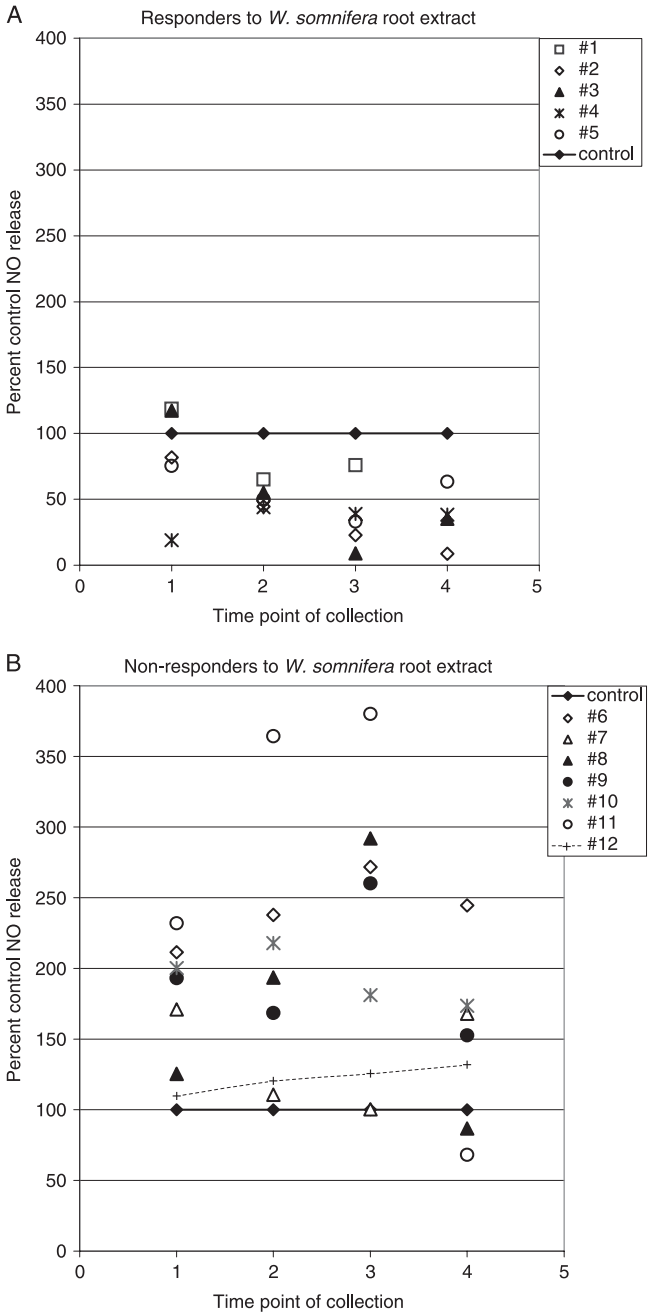

Figure 2. (A) Responders to *W. somnifera* root extracts. *W. somnifera* root extracts caused a statistically significant, long-term, decrease in NO released by cartilage explants from 5 of 11 OA patients (OA 1–5) at time points 2, 3 and 4. Levels of NO released by control explants are normalized to 100% (solid line). (B) Non-responders to *W. somnifera* root extracts. *W. somnifera* root extracts caused a statistically significant, long-term increase in levels of NO released by cartilage explants from 6 of 11 OA patients (OA 6–11) at all time points. The dotted line (+) indicates a significant increase in levels of GAGs released by the same six cartilage explants at time points 2, 3 and 4. Levels of NO and GAG released by control explants are normalized to 100% (solid line).

drug treated explants were tested for statistical significance at the 95% confidence level using the Student's two-tailed *t*-test for paired samples.

Magnitudes of NO and GAG release by drug treated explants are expressed as mean percent of NO or GAG released, with respect to levels of NO and GAG released by controls (cartilage explants at each time point, for each patient, without drug treatment). High standard deviations of these mean values were due to patient–patient variation. Therefore, statistically significant versus insignificant data are shown clearly.

RESULTS

Rationale for the experimental design of this study

Since *W. somnifera* root extract is known to be an immunomodulatory agent (Bani *et al.*, 2006), its anti-inflammatory potential was assessed by measuring levels of NO released by human OA cartilage explants treated with/without *W. somnifera*. GAIT, the large-scale, multicentre clinical trial which tested the effects of dietary glucosamine hydrochloride (glucosamine) and sodium chondroitin sulphate (CS) for treatment of knee osteoarthritis, conclusively showed that glucosamine caused a statistically significant decrease in joint pain in a certain subset of osteoarthritis (OA) cases. This suggested that glucosamine may regulate the levels of nitric oxide (NO) released by human OA cartilage. Since few studies have examined the effects of glucosamine sulphate (GlcS) on human OA cartilage *in vitro*, GlcS was also included in this study.

In this context, the antiinflammatory effects of *W. somnifera* and glucosamine sulphate were evaluated by assaying levels of NO released by explants of human OA cartilage treated with/without the two drugs. Glycosaminoglycan (GAG) release from these same explants was also measured to evaluate the chondroprotective potential of each drug. Then, it was determined whether there was a correlation between the chondroprotective and antiinflammatory effects of each drug. This approach allowed comparison of two major drugs (*W. somnifera* and glucosamine sulphate) with proven antiarthritic activities in animals and humans.

Effects of glucosamine sulphate (GlcS) on NO and GAG release from OA cartilage

Responders. Figure 1 shows the effects of GlcS on the levels of GAGs and NO released by OA cartilage explants from 5 of 11 patients tested. When compared with the controls, the GlcS treated explants released 42.56 ± 26.78% and 56.68 ± 19.10% of levels of GAG and NO, respectively, at time point 1. At time point 2, GlcS treated explants released 65.27 ± 25.36% of levels of GAG released by the corresponding controls. Statistical analysis showed that GlcS induced reductions in GAG released by explants at time points 1 and 2, were significantly below that of the corresponding controls. However, the GlcS induced reduction in NO released by these explants was statistically significant at time point 1 only.

At later time points, the GlcS induced decreases in GAG and NO released by explants lacked statistical significance. Thus, at time points 3 and 4, GlcS treated explants released 35% and 10% less GAG, respectively, when compared with the levels of GAG released by controls (Fig. 1: black versus grey bars). Similarly, GlcS treated explants from the five responders released 15%, 20%, and 28% less NO respectively; when compared with the levels of NO released by controls at time points 2, 3 and 4 (Fig. 1 black versus white bars).

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Table 1. Comparative effects of glucosamine and *W. somnifera* extracts on OA cartilage

	W. somnifera root extract		Glucosamine sulphate (GlcS)	
Activity	Responders	Non-responders	Responders	Non-responders
Antiinflammation	Patient 1, 2, 3, 4, 5 Long-term	Patient 6, 7, 8, 9, 10, 11	Patient 2, 4, 6, 8, 11 Short-term	Patient 1, 3, 5, 7, 9, 10
Chondroprotection	Patient 1, 2, 3, 4, 5 Absent	Patient 6, 7, 8, 9, 10, 11	Patient 2, 4, 6, 8, 11 Short-term	Patient 1, 3, 5, 7, 9, 10

Chondroprotective and antiinflammatory effects of glucosamine (GlcS) and *W. somnifera* in cartilage explants from 11 OA patients (OA 1–11) are shown. Chondroprotective and antiinflammatory effects measure statistically significant decreases in GAGs and NO release, respectively, by OA cartilage explants. Explants from patients (2, 4, 6, 8, 11) gave chondroprotective and antiinflammatory responses to GlcS (responders). Explants from patients (1, 2, 3, 4, 5) gave an antiinflammatory response (but no chondroprotective response), to *W. somnifera.*

Common response: Both drugs induced an antiinflammatory response in patients 2 and 4 (underlined). Explants from patients 7, 9 and 10 were non-responders to both drugs (underlined numbers in italics).

Differential response: Explants from patients 6,8,11 showed an antiinflammatory response to GlcS, but not to *W. somnifera.* Conversely, explants from patients 1, 3 and 5, showed an antiinflammatory response to *W. somnifera* extract, but not to GlcS.

Therefore, GlcS induced significant, short-term, chondroprotective and antiinflammatory effects in cartilage explants from these five patients (OA 2, 4, 6, 8, 11) *in vitro*. These patients were termed as 'responders' to GlcS *in vitro* (Table 1).

Non-responders. In explants from the remaining 6 of 11 patients, (OA 1, 3, 5, 7, 9, 10), GlcS induced a statistically significant increase in NO release at time points 1 and 4 (143.36 ± 77.43% and 155.46 ± 68.70%) respectively, with respect to NO levels released by the corresponding controls. However, GAG release was not significantly altered in these GlcS treated explants at any time point. These results suggest that cartilage explants from patients in this second subset did not show a chondroprotective response to GlcS. Therefore, these patient samples were termed 'non-responders' to glucosamine.

To summarize, it was found that OA cartilage explants displayed two types of response to GlcS. In 'responders', GlcS induced statistically significant, short-term, chondroprotective and antiinflammatory effects. In non-responders, GlcS caused a statistically significant increase in NO release, without affecting GAGs release.

The existence of responders and non-responders to GlcS has been reported Thus, Dodge and Jimenez (2003) showed that chondrocytes from 40% of human OA cases did not show a chondroprotective response to GlcS. Specifically, GlcS treatment did not increase proteoglycan content and failed to decrease levels of the matrix metalloproteinases secreted by these OA chondrocytes. In this context, the data on 'responders' and 'non-responders' to the chondroprotective effects of glucosamine (Fig. 1) is not surprising. This is because osteoarthritic cartilage from patients during knee replacement surgery, may/may not exhibit chondroprotective responses to drugs.

Effects of *W. somnifera* root on NO and GAG release from OA cartilage

Responders. Figure 2A shows the effects of *W. somnifera root* extract on NO release in cartilage explants from 5 of 11 OA patients (OA 1–5). Explants from five patients treated with *W. somnifera* extract released 53.89 ± 14.97%, 50.20 ± 22.67 and 30.79 ± 23.19%, respectively, of NO levels released by the controls at time points 2, 3 and 4, statistical analysis showed that these values were significantly below controls, i.e. *W. somnifera* root extract caused a significant, 50–70% sustained reduction in levels of NO released by explants from these five patients. These data suggest that *W. somnifera* extract had antiinflammatory activity in cartilage explants from 5 of 11 OA patients tested. This subset of patients may be considered as 'responders' to *W. somnifera* (Table 1).

Treatment with *W. somnifera* extract did not significantly alter the levels of GAG released by explants from the 'responders' (data not shown).

Non-responders. Figure 2B shows that *W. somnifera* extract induced a statistically significant, long-term increase in NO release by OA cartilage explants from the remaining six patients (OA 6–11). Magnitudes of NO release ranged from 191.09 ± 46.30%, 210.70 ± 68.33%, 234.20 ± 91.0% and 154.90 ± 79.80% of controls at time points 1, 2, 3 and 4, respectively. Statistical analysis showed that these levels of NO release were significantly higher than the levels of NO released by control explants at these time points. Thus, during 8 days of culture, *W. somnifera* extract caused a significant and sustained 55–135% increase in NO released by cartilage explants from these six OA patients. Interestingly, *W. somnifera* treatment of cartilage explants from this subset of six patients, also resulted in a statistically significant, 20–25% increase in levels of GAG released with reference to controls, at time points 2, 3 and 4 (Fig. 2B dotted line). Therefore, in this subset of patients, there was a direct correlation between the increased release of NO and GAG in response to treatment with *W. somnifera* extracts, suggesting that *W. somnifera* enhanced the loss of GAGs from the matrix of these OA cartilage explants. Indeed, these patients may be considered as 'non-responders' to *W. somnifera*, a concept consistent with Ayurvedic practice (in Ayurveda, only certain OA patients would be treated with *W. somnifera* root extracts).

In summary, *W. somnifera* root extracts exhibited strong, long-term, antiinflammatory potential in cartilage explants from 'responders' (50% of OA patients).

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However, this herbal drug also induced a significant increase in levels of NO and GAGs released by explants from 'non-responders'. The relationship between chondroprotective and antiinflammatory effects of *W. somnifera* root and glucosamine is discussed below.

Comparative effects of *W. somnifera* root and glucosamine sulphate on NO and GAG release from OA cartilage

Figures 1 and 2 showed the quantitative effects of *W. somnifera* root and glucosamine sulphate on cartilage damage. Table 1 summarizes these data in a qualitative manner in order to compare and contrast the effects of the two drugs on cartilage damage.

Both drugs (0.05 mg/mL w/v) significantly reduced NO release by cartilage explants from 50% of the 11 cases tested. However, this antiinflammatory effect was long-term in the case of *W. somnifera* root and short-term for GlcS (Figs 1, 2A and Table 1). In the case of GlcS, it was observed that explants from 'responders' showed chondroprotective and antiinflammatory responses to this nutraceutical. However, cartilage explants of responders to *W. somnifera,* showed an antiinflammatory 'responders' without a concomitant chondroprotective response to this herbal rasayana.

For each drug, there were two distinct subsets of patients' cartilage samples, in terms of a beneficial antiarthritic response ('responders' versus 'non-responders'). Table 1 also shows that OA cartilage from patients showed differential and opposing responses to the chondroprotective and antiinflammatory effects of **GlcS** and *W. somnifera in vitro*, i.e. some OA patient samples showed common responses to both drugs, whereas the others showed opposing responses to these drugs *in vitro*.

Relationship between chondroprotective and antiinflammatory activity

As mentioned, the antiinflammatory effects induced by GlcS (Fig. 1) were associated with chondroprotective responses in explants of patients termed 'responders'. However, this was not the case with *W. somnifera* (Fig. 2A). The differential effects of these two drugs may be due to the two following reasons.

First, the lack of a chondroprotective response in *W. somnifera* treated explants (in responders), may occur because reduction in NO release is necessary, but not sufficient; for chondroprotection. Thus, inhibition of proteases and/or cytokines within the cartilage matrix may also be required before these OA cartilage explants show reduced GAG release. Second, levels of endogenous cytokines in the cartilage matrix can differentially modulate the antiinflammatory effects of *W. somnifera* with respect to turnover of GAGs in the cartilage matrix. This point is supported by a comprehensive study by Dingle (1991). After examining the actions of 13 different non-steroidal antiinflammatory drugs (NSAIDS) in 830 patients, these researchers found that the NSAIDS may be divided into three categories based on their *in vitro* action on the extracellular matrix of human arthritic cartilages: Certain NSAIDs (e.g. aceclofenac) stimulated matrix synthesis, whereas others (e.g. aspirin) did not significantly affect matrix synthesis. Ibuprofen and nimezulide fall into the third group of NSAIDS, which significantly inhibited matrix synthesis. The authors concluded that these three different effects of NSAIDS on GAG synthesis may reflect the ability of NSAIDS to inhibit locally produced pro-inflammatory cytokines such as IL-1 (Dingle, 1999).

Although Dingle did not discuss the existence of 'responders' versus 'non-responders' to a given NSAID, the results clearly show that antiinflammatory agents (such as NSAIDS), have varying and even opposite effects on GAGs synthesis and turnover. Similarly, the data show that the antiinflammatory activity of glucosamine in 'responders' (Fig. 1, Table 1), was associated with chondroprotective effects (decreased GAG release). In contrast, the antiinflammatory activity of *W. somnifera* was not associated with chondroprotective effects (Fig. 2A and Table 1).

DISCUSSION

An *in vitro* model to measure the effects of *W. somnifera* root extract and glucosamine on OA cartilage damage was validated. As reported earlier, the responses of OA cartilage explants to nutraceuticals fell into two subsets: 'responders' versus 'non-responders' *in vitro* (Sumantran *et al.*, 2007a and b). Before we discuss the present data, we provide a scientific rationale for statistical analysis of subsets of data.

Rationale for statistical analysis of subsets of data

While a strict statistical approach demands averaging data on the effects of a drug on all OA patient samples, data of subsets of patients were analysed for two important reasons. First, the OA cartilage used was discarded from joints of chronic OA patients undergoing knee replacement surgery. Such tissue cannot be considered as 'random samples', as only some of these patients may have responded to antiarthritic drugs.

Second, the phenomenon of a partial response to chondroprotective drugs such as glucosamine was proven in the recent GAIT study. GAIT is the first, large-scale, multicentre clinical trial in the USA to test effects of dietary glucosamine hydrochloride (glucosamine) and sodium chondroitin sulphate (CS) for treatment of knee osteoarthritis (NCCAM, 2006). The study found that glucosamine and CS provided statistically significant pain relief in the subset of OA patients with moderate-to-severe pain. However, these nutraceuticals were ineffective in the subset of patients with mild pain.

Effects of glucosamine sulphate on OA cartilage damage

While others have documented the existence of responders and non-responders to the chondroprotective effects of GlcS *in vitro* (Dodge and Jimenez, 2003), this study is the first to show a similar pattern with respect to antiinflammatory effects of glucosamine sulphate in human OA cartilage *in vitro*. Moreover, statistically significant differences were shown between responders

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and non-responders with respect to GAGs and NO released by glucosamine treated OA cartilage explants.

The literature reports conflicting data on the effects of glucosamine sulphate (GlcS) on the levels of nitric oxide (NO) released by human OA cartilage *in vitro.* One study showed that GlcS did not significantly alter NO levels in cultured human OA chondrocytes (Pipierno *et al.*, 2000). Another study showed that glucosamine hydrochloride partly suppressed NO production in human OA chondrocytes (Nakamura *et al.*, 2004). Our report is consistent with both studies, in that there were both responders and non-responders to the antiinflammatory effects of glucosamine sulphate *in vitro*.

Effects of *W. somnifera* root extract on OA cartilage damage

W. somnifera root extracts caused statistically significant, sustained, antiinflammatory effects in cartilage explants from 50% of patients (Fig. 2A: responders). This is the first study showing direct, statistically significant, antiinflammatory effects of *W. somnifera* on human OA cartilage *in vitro*. These results are consistent with reports showing immunomodulatory and antiarthritic actions of *W. somnifera* in animal and human trials (Ganju *et al.*, 2003; Mishra *et al.*, 2000). Cartilage explants from the remaining OA patients (Fig. 2B) were non-responders to *W. somnifera* extract.

Responders versus non-responders

There are few studies documenting the existence of responders versus non-responders to the chondroprotective and antiinflammatory effects of antiarthritic drugs *in vitro*.

Four reports suggest that growth factor resistance may be associated with the partial response to such antiarthritic drugs. Thus Loeser *et al.* observed that human and monkey OA chondrocytes staining positive for nitrotyrosine or IL-1beta, were largely resistant to IGF-1, suggesting that NO-induced oxidative stress can alter sensitivity to this growth factor (Loeser *et al.*, 2002). Using *in vitro* cultures of human cartilage explants, Ismaiel *et al.* observed a partial response to the degradative effects of IL-1. While arthritic cartilage was more susceptible to the degradative effects of IL-1 than normal cartilage, they concluded that 'cartilage explants from some individuals are susceptible to the degradative effects of IL-1 whereas others are refractory' (Ismaiel *et al.*, 1992). A third report showed that after prolonged exposure to IL-1beta, human OA chondrocytes develop selective tolerance involving NO and PGE(2) release but not MMP-13 production, metabolic activity or matrix metabolism (Lee *et al.*, 2002). In this context, the levels of expression of different growth factors, cytokines and their receptors, in OA cartilage matrix may account for refractoriness/resistance of certain patients' cartilage to the chondroprotective and anti-inflammatory effects of glucosamine and *W. somnifera*.

In addition to growth factor resistance being associated with a partial response to antiarthritic drugs, signaling events downstream of NO release must also be considered. In animal models of joint disease, Jouzeau *et al.* proved that the inhibitory potency of IL-1-beta on proteoglycan synthesis and its stimulating effect on COX-2 activity, depend both on NO and superoxide production within the cartilage matrix (Jouzeau *et al.*, 2002). The inhibition/lack of inhibition of PGE_2 release and/or COX-2 activity also significantly contributed to a partial response of arthritic cartilage to antiinflammatory drugs (iNOS inhibitors) and antioxidants (*N*-acetylcysteine) *in vitro* (Mathy-Hartet *et al.*, 2002).

Future studies on the expression of additional markers for cartilage damage (iNOS, prostaglandin E2, Cox-2, apoptosis markers) will elucidate the downstream effects of the observed changes in NO and GAGs released by OA cartilage in the responders versus non-responders to *W. somnifera* and glucosamine treatment.

CONCLUSIONS

The results are novel and physiologically relevant for three important reasons. First, knee cartilage from chronic OA patients was used, since any antiarthritic drug must be effective on degenerating cartilage. Second, to mimic Ayurvedic tradition, aqueous extracts of drugs were used. Third, standard serum containing growth media for cartilage culture was used. Thus, the observed differential effects of *W. somnifera* root and glucosamine on OA cartilage damage are likely to reflect their *in vivo* activities.

In summary, this *in vitro* model of human cartilage damage quantitatively measures statistically significant differences between responders and non-responders to antiarthritic drugs. Such an approach provides important and realistic preclinical data on antiinflammatory and chondroprotective activities of new and existing drugs.

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