Antibacterial activity of glucosamine sulfate and chondroitine sulfate?

Sirs,

Osteoarthritis (OA) remains one of the most common forms of musculoskeletal disease. Eighty percent of patients have limitation of movement, and 25% cannot perform major daily activities. Glucosamine sulfate (GS) and chondroitine sulfate (CS) were shown to delay x-ray progression of OA (1). Glucosamine chloride along with CS was shown to reduce symptoms of moderate-severe painful knee OA (2). GS and CS supplements declined 5-year operative risk after its discontinuation (3). The mechanism of action of this therapy is still unknown. Recent data demonstrating the efficacy of co-trimoxazole administered as prophylaxis for urinary tract infections in relieving symptoms of patients with knee OA have raised the possibility of participation of the fecal flora in pathogenesis of OA (4, 5). We decided to examine the antibacterial activity of GS, CS separately and both in one solution and as a trademark compound Megagluflex on E. coli growth *in vitro*. Working solutions of GC- [Megagluflex (MGF), "American Health", NY)] in concentration ranging from 40mcg/ml to 100mg/ml and glucosamine sulfate (GS) (Sigma), chondroitine sulfate (CS) (Sigma) (1mg/ml, 50mg/ml) were prepared in normal saline as dissolvent. Inoculums of up to 10^4 /ml E. coli (strain ATCC 25922) was prepared in serum supplemented Brain heart growth media. One ml of the inoculum solution was mixed with 0.2 ml of different concentration of GC, GS, CS solutions or with control normal saline and incubated in at 37°C for 16 hours. The number of colonies was counted. pH testing was performed for every mixture. We examined antibacterial properties of solutions with components of MGF: vitamin C and $MnSO_4$ (0.166mg/ml, Riedel-De Haen) and solutions with similar pH (5.0) (HCL diluted by normal saline), osmolality (933mOsm/kg) (glucose solution with normal saline) like that of the tested MGF solution (Table I). Experiments

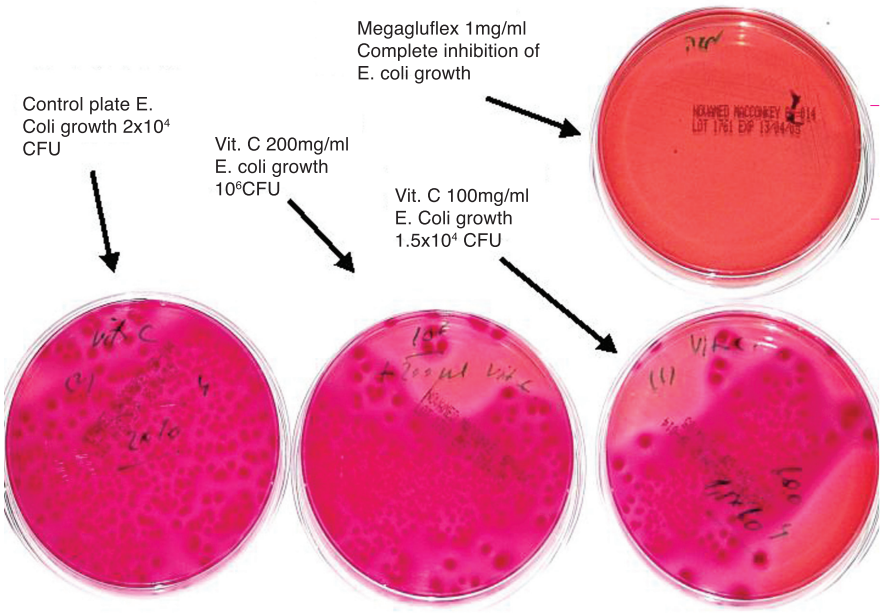

Fig. 1. Complete inhibition of E. coli growth by Megagluflex solution (1mg/ml) is seen (right upper plate) compared to control plate (left at lower range) and solutions of vitamin C (mid and right at lower range).

Table I. Growth of E. coli ATCC 25922 in control media and in media supplemented with Megagluflex (MGF), glucosamine sulfate (GS), chondroitine sulfate (CS), $MnSO_4$, HCl and glucose solutions.

Nutritional supplement Date of experiment	Concentration of the nutritional supplement (mg/ml)	Time-0 No of CFU	Time-0 No of CFU with MGF	No of CFU after 16 hours	No of CFU after 16 hours with MGF	Inhibition of E. coli growth
Megagluflex						
8.08.2005	40mcg/ml	$4X10^4$	$1.5X10^4$	10^8	10^6	Mild
8.08.2005	200mcg/ml	$4X10^4$	$1.5X10^4$	10^8	10^6	Mild
25.01.2005	1mg/ml	$3X10^2$	$2.5X10^2$	$2X10^7$	$<10^2$	Severe
21.02.2005	1	$3X10^2$	$2.5X10^2$	$2X10^7$	$<10^2$	Severe
30.05.2005	4	$3X10^2$	$1.5X10^2$	$2X10^6$	$<10^2$	Severe
8.08.2005	1	$4X10^3$	$4X10^3$	$2X10^7$	10^5	Mild
21.08.2005	100	$4X10^2$	$5X10^2$	$2X10^7$	$1.2X10^2$	Severe
26.09.2006	4	$3X10^4$	$8X10^3$	10^6	$2X10^6$	No
3.10.2006	1	$4X10^4$	$5X10^4$	10^6	10^6	No
3.10.2006	4	$5X10^4$	$5X10^4$	10^6	10^6	No
12.11.2006	100	$3X10^3$	$6X10^4$	10^6	$9X10^2$	Severe
27.11.2006	100	$3X10^3$	$3X10^3$	$2X10^5$	10^2	Severe
5.12.2006	100	$2X10^3$	$5X10^4$	10^6	$2X10^2$	Severe
p	for MGF	concentration	>1mg/ml			0.001
GS/CS/OS						
5.02.2006	1/1/1	$3X10^2$	$2.5X10^2$	$5X10^7$	$10^7/10^7$/ND	No
5.02.2006	50/50/50	$3X10^2$	$2.5X10^2$	$5X10^7$	$10^7/10^7$/ND	No
20.02.2006	50/50/50	$3X10^2$	$2.5X10^2$	$5X10^7$	$2.5X10^5/2.5X10^5/3X10^5$	Mild
21.03.2006	50/50/50	$3X10^2$	$1.5X10^2$	$5X10^7$	$10^2/5X10^7/3X10^7$	Severe/No/No
19.04.2006	50/50/50	$4X10^3$	$4X10^3$	$5X10^7$	$2X10^7/5X10^7/2X10^7$	No
Manganese sulfate	0.166	$3X10^3$	$2.5X10^2$	$5X10^7$	$4.5X10^7$	No
HCl	pH 5.0	$2.5X10^2$	$2.0X10^2$	$5.5X10^7$	$4X10^7$	No
Glucose	933mOsm/kg	$3X10^2$	3.010^2	$4.5X10^7$	$3X10^7$	No

MGF: Megagluflex; CFU: colony-forming unit; GS: glucosamine sulfate; CS: chondroitine sulfate; OS: glucosamine sulfate and chondroitine sulfate in one solution; ND: not done; No: no inhibition of E. coli growth; Mild: mild inhibition of E. coli growth; Severe: severe inhibition of E. coli growth.

with 3 repeatable results were taken for consideration. MGF inhibited E. coli growth significantly (p=0.001) in MIC of 1mg/ml and higher (Table I, Fig. 1). Close to expired time of the drug antibacterial activity declined and persisted at concentration of 100 mg/ml. Solutions of GS and CS separately and in one solution mildly inhibited E. coli growth in one of 5 experiments only in concentration of 50mg/ml. Solutions of vitamin C and manganese sulfate, the components of Megagluflex, and control media, such as control solutions with pH and osmolality like that of MGF solution did not affect E.coli growth. MGF, GS, CS solutions were negative for bacterial contamination. Several theories have been proposed for explanation of GC efficacy. GS is considered to be a precursor of the aggrecane molecules of the joint cartilage. Stress protective activity of GC has been recently reported to increase the cartilage tissue resistance to mechanical, physical and chemical agents (6). The drug was also shown to display antioxidant properties (7). Some arthritogenic E. coli and other enterobacteriaciae have been implicated in the development of spondyloarthropathies and other inflammatory arthritides. OA is accompanied by inflammatory manifestations. Expression of Toll-like receptors 2 and 4 [lipopolysaccharide (LPS)-binding] has recently been found up-regulated in lesion areas of OA cartilage (8). Innate production of tumor necrosis factor-alpha and interleukin-10 upon LPS-stimulation has been associated with radiological progression of knee osteoarthritis (9). Bacterial endotoxin (LPS) promotes the synthesis of LPS-binding protein (LBP), and forms a LPS-LBP complex that binds to LPS receptor CD14. Endotoxin-CD 14 signaling triggers a cascade that leads to inflammatory cytokine production (10). Our data suggests that MGF trademark compound has certain antibacterial activity against E. coli *in vitro*. Sustained antibacterial activity of GS, CS of another manufacturer in a separate and one solution was not found. Further trials are needed to clarify the antibacterial activity of GC.

A.P. ROZIN[1], *MD*
M. GOLDSTEIN[2],
H. SPRECHER[2], *MD, PhD.*

[1]B. Shine Department of Rheumatology and [2]Department of Microbiology, Rambam Health Care Campus and Technion, Haifa, Israel.

Address correspondence to: Dr. Alexander Rozin, B. Shine Department of Rheumatology Rambam Health Care Campus, POB 9602, Haifa 31096, Israel.
E-mail: a_rozin@rambam.health.gov.il

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