COMMUNICATION

Failure of Rumalon to Increase Sulfate Incorporation by Articular Chondrocytes in Monolayer Culture

Sir:

Several tissue extracts are reported to increase the incorporation of sulfate by cartilage in vitro (1). Rumalon, a proprietary extract of bovine rib cartilage and bone marrow which has received numerous clinical trials in the treatment of osteoarthritis, is said to do the same (2). The preparation has been found to increase mucopolysaccharide production by mouse vertebral chondrocyte cultures (3) and to retard fibroblastic dedifferentiation of chick embryo chondrocytes in monolayers (4, 5). Our studies of articular chondrocytes and skin fibroblast monolayer cultures fail to support these findings and we think it worthwhile to place these observations on record.

The experiments were carried out on secondary cultures of articular chondrocytes and skin fibroblasts from 2 rabbits about 10 weeks old using methods described previously (6, 7). A metacresol-free lot of Rumalon (no. 04101) was received through the generosity of Dr. W. P. Koella, Robapharm, Basel. According to data provided by the distributor, the inorganic sulfate content was 5.6 mg%; the vehicle, water; the osmolar freezing point depression $\Delta t = -16°C$. The culture medium was Dulbecco's, changed by substituting $MgCl_2$ for $MgSO_4$ so that it contained no inorganic sulfate. Adjustments were made to keep the several media isotonic and containing equal concentrations of inorganic sulfate. Thus, 0.15% Na_2SO_4 and 23.8% NaCl in Dulbecco's medium were added to 100 ml of the media in the quantities indicated in Table 1.

The cells were inoculated into Falcon flasks with the media indicated in Table 1 as well as medium containing 2% Rumalon that had been immersed in a boiling water bath for 15 minutes. The cells were exposed to the test media for six days (fed day 0, 3, and 5). On the day 5 feeding, $Na_2\ ^{35}SO_4$, 1.4 μCi/ml was added to the culture medium. The cells were harvested 20 hours later. The medium was dialyzed and counted by the methods we have already described. The DNA content of the cell pellet was measured by the method of Burton, somewhat modified (7). The values obtained were as indicated in Table 2.

In trying to explain the disparity between these and the published data, a question arises about the differentiation of these cells; were they capable of responding? The cultured chondrocytes probably have been altered from their native state but they do retain a unique capacity for making sulfated macromolecules (6). Relative to rabbit articular chondrocytes considered as unit value, dpm $^{35}SO_4/\mu g$ DNA for other cell types are: human articular

Table 1. Composition of Culture Media

Composition	Control	Rumalon 1% v/v	Rumalon 2% v/v
Dulbecco's medium (ml)	90	90	90
Fetal calf serum (ml)	10.0	10.1	10.2
23.8% NaCl (λ)	0	25	50
Rumalon (ml)	0	1.0	2.0
0.15% Na_2SO_4 (λ)	86	42	0
SO_4 from Dulbecco's medium (mg/liter)	0	0	0
SO_4 from 10% fetal calf serum (mg/liter)	9.33	9.32	9.31
SO_4 from penicillin-streptomycin (mg/liter)	2.00	1.98	1.96
SO_4 from carrier (mg/liter)	0.21	0.21	0.21
SO_4 from Rumalon (mg/liter)	0	0.55	1.09
SO_4 from added Na_2SO_4 (mg/liter)	0.86	0.42	0.00
Total inorganic SO_4 (mg/liter)	12.40	12.48	12.57

Table 2. Effect of Rumalon on Cultured Cells

Cell type	Experimental medium	DNA (μg/flask)*	$^{35}SO_4$ (10^4 dpm/μg DNA)*
Chondrocyte	Control	28.5 ± 0.77	3.25 ± 0.11
		30.6 ± 0.23	2.48 ± 0.06
Chondrocyte	1% Rumalon	28.4 ± 1.00	3.09 ± 0.11
		30.6 ± 1.50	2.19 ± 0.11
Chondrocyte	2% Rumalon	27.7 ± 1.13	2.71 ± 0.12
		29.9 ± 1.00	2.19 ± 0.08
Chondrocyte	2% Rumalon, heated	27.8 ± 0.61	2.90 ± 0.10
		29.8 ± 0.54	2.29 ± 0.09
Fibroblast	Control	32.5 ± 0.90	0.692 ± 0.03
Fibroblast	1% Rumalon	34.6 ± 1.11	0.566 ± 0.03
Fibroblast	2% Rumalon	34.7 ± 0.75	0.623 ± 0.02
Fibroblast	2% Rumalon, heated†	31.6 ± 0.42	0.688 ± 0.04

* Mean ± standard error; 8 flasks in each group except †, 7 only.

chondrocytes, 1.18 ± 0.23; rabbit skin fibroblasts, 0.25 ± 0.01; VERO, 0.033 ± 0.003; HeLa, 0.013 ± 0.003; N19-31, 0.005 ± 0.0002. Most of the counts in the chondrocyte media are made dialyzable by digestion with testicular hyaluronidase but not by dilute NaOH or pronase. The presumption then is that the cell product contains chondroitin sulfate. Variation in the amount of this product has resulted from other agents tested under similar circumstances (7).

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