

AGING AND MECHANOSENSITIVITY OF HUMAN BONE CELLS

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Introduction

With increasing age the human skeleton decreases in density, thereby compromising its load bearing capacity. Bone tissue adapts to mechanical loading. However an age dependent decrease of adaptation has been recently described in rats (1). We therefore examined whether age-related differences exist in growth potential of human bone cells, and in their response to mechanical stress by fluid flow in vitro.

Materials and methods

Human primary bone cells were obtained as outgrowth from transiliac bone biopsies of 31 donors (males and females) without metabolic bone disease, who entered the hospital for maxillofacial or orthopaedic surgery, with an age range from 7 to 90 years (mean \pm SEM, 29 ± 6 years). They were grown to confluency, passaged, plated at 5×10^5 cells/glass slide, and treated for 1 h with or without mechanical stress by pulsating fluid flow (PFF, 0.7 Pa, 12 Pa/sec, 5 Hz) in DMEM with 2% FBS. Then, cells were post-incubated in fresh medium for 24 h without PFF. Medium prostaglandin E₂ (PGE₂) and 6-keto-PGF_{1 α} (the stable metabolite of PGI₂) concentrations were quantitated by immuno-assay.

To test their osteoblastic phenotype, cells were incubated for 3 days with or without 10^{-8} M 1,25(OH)₂D₃. Osteocalcin was measured in the conditioned medium by radioimmunoassay. The protein content of the cell layer was measured using a BCA protein assay reagent kit. All results were analysed by Wilcoxon's signed-rank test, and a *p*-value of <0.05 was considered significant. Correlations were analysed by linear regression.

Results

The time needed for cell cultures to reach confluency almost doubled between 7 and 90 years of age (Table 1).

Table 1: Effect of donor age on cell outgrowth and cell growth rate of bone cell cultures.

age group (yrs)	age range (yrs)	n (k/w)	d.0 to cell outgrowth (days)	d.0 to 1 st passage (days)	1 st passage to 2 nd passage (days)
<20	7-13	15 (6/9)	5 ± 2	14 ± 3	7 ± 0
20-50	28-44	3 (0/3)	8 ± 1	16 ± 3	10 ± 3
50-70	55-59	3 (2/1)	7 ± 4	20 ± 7	8 ± 2
>70	72-90	7 (5/2)	$9 \pm 2^{**}$	$21 \pm 7^*$	$13 \pm 2^{**}$

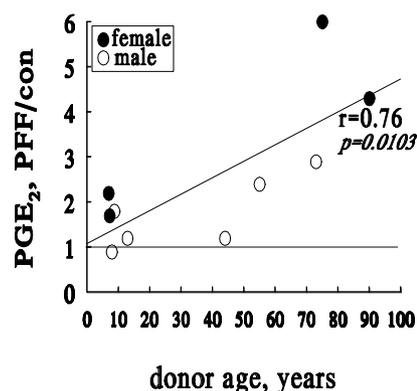
After the cell monolayer from the bone fragments reached confluency, cells were trypsinized (1st passage) and plated at 25×10^3 cells per well in 6-well culture dishes. At subconfluency, cells were trypsinized again (2nd passage), and plated on glass slides for PFF treatment the following day. Data are mean \pm SEM. * Significantly different from age group <20 years, *p*<0.05, ***p*<0.01.

The first visible outgrowth of cells from the cultured bone chips occurred earlier in cultures from children than those from adults. Also, the culture time needed between seeding of the bone chips and first and second passage increased with age, suggesting an age-related decrease in the growth rate of the bone-derived cell population. The total growth period from day 0 to the second passage was correlated with age in both bone cells from female (*r*=0.74; *p*=0.041) and male (*r*=0.79; *p*=0.0005) donors. This diminished growth capacity may be related to the increased time needed for bone healing in older donors.

There was no significant correlation between donor age and basal osteocalcin release, or between donor age and the magnitude of the osteocalcin response to 1,25(OH)₂D₃, suggesting that the osteoblastic characteristics of the cultures did not vary with age.

Medium PGE₂ and PGI₂ levels increased with mechanical stress by PFF within 5 minutes, and were 1-6 fold (PGE₂) and 1-3 fold (PGI₂) higher after 1 h PFF than in non-stressed controls. During the 24 h post-PFF period, both PGE₂ and PGI₂ production were still increased. The PFF-mediated prostaglandin upregulation was positively correlated with donor age (Figure 1). Cell cultures from older donors showed a higher response to PFF, both during and after PFF treatment, than cells from younger donors. Roughly, the magnitude of the response tripled between 7 and 90 years of age.

Figure 1: Regression of donor age in relation to treatment with PFF on prostaglandin release by human bone cell cultures.



Discussion

A possible explanation for the finding that cell cultures from older donors showed a higher response to PFF than cells from younger donors may be found in differences in the cell population obtained from young and old donors. As shown in Table 1, the growth of bone cells from collagenase-treated trabeculae was faster in children than in older donors, leading to a doubling of the time needed to reach confluency between 7 and 90 years. This suggests a loss of immature bone cells, with a high proliferative capacity, with age.

We conclude that the growth potential of bone tissue diminishes with donor age, but the response of the bone cell cultures to mechanical stress increases. Since mature bone cells are more sensitive to PFF than immature cells (2), the correlation of the prostaglandin response with donor age suggests that the cell cultures from aged donors represent more mature cells than those from young donors, in line with their diminished growth potential. Thus the loss of bone with increasing age may be related to an age-dependent loss of bone growth potential, rather than a decreased bone cell response to mechanical stress.

References

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- Turner C.H. et al., J Bone Miner Res 10:1544-1549, 1995.

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