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Investigation of Effect of Various Agents on Periosteal Bone Formation

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ABSTRACT

Periosteal bone formation is certainly influenced by several factors. The present investigation shows that immobilization in plaster for a short time has no notable effect on periosteal bone formation after traumatization of the marrow. On the other hand, the traumatization of the animal by daily tube feeding reduces periosteal bone formation markedly, possibly owing to a stress reaction. Tanderil given by feeding tube appears to diminish the reduction of bone formation due to tube feeding alone. Phenytoin given by tube produces no change in the effect of tube feeding alone. Anabolic steroids given as a single injection during the period in question produce a non-significant increase in periosteal bone formation.

INTRODUCTION

An experimental design Abstract (1), in which the periosteal bone formation initiated by evacuation of marrow was used as a parameter, was described in a previous paper. The results were analysed statistically and satisfied the demands that may reasonably be placed on a biologic method. The method may therefore be regarded as acceptable. The main purpose of the present investigation was to evaluate the sensitivity of the method in various respects. The investigation was therefore extended to include:

1. Evaluation of the role played by inactivity. In a control group one of the legs was only placed in plaster (group A). In the main group the evacuation of the marrow was supplemented by treatment with plaster (group B).

2. Evaluation of the effect of tube feeding *per se* (group C).

3. Effect of Tanderil[1] given by feeding tube (group D).

4. Effect of Difhydan[2] given by feeding tube (group E).

5. Effect of Tanderil given by injection (group F) and pertinent control group with solvent for Tanderil (group G).

6. Effect of Anadur[3] given by injection 5 days before operation (group H).

MATERIAL

Each experimental group consisted of 17–26 male rabbits of a brown Swedish land breed (groups A, B, C, D, E, H) or albino rabbits (groups F and G), weighing between 1.8 and 2.2. kg. Each breed was obtained from a separate dealer and for at least 1 week the animals were kept in quarantine in cages in which they were also kept during the rest of the experiment.

Details of the operative method used for evacuation of marrow and calculation procedure is given in the previous paper (1).

STATISTICAL METHODS

The fundamental statistical method is based on the difference between periosteal, new-formed bone on the operated, and on the unoperated, tibia. In order to increase the reliability of the measurements of the amount of new-formed bone further, double and triple determinations were made at different levels of the operated and the unoperated side (see Table 1). The triple measurements did not produce any improvement of the reliability, and it was therefore decided to measure only 2 sections from different levels, but only in 2 of the groups. Measurements of the newly-formed bone surfaces made by 2 different persons were also compared (Table 1).

[1] Tanderil Geigy (= oxiphenbutazone).

[2] Difhydan Leo (= phenytoin).

[3] Anadur Leo (active substance Nortestosteroni 3-) (4-Hyxyl-oxiphenyl)-propion.

Table I. *Error of method*

Reproducibility measured as amount of new-formed bone determined by comparison of findings in sections from different levels of same bone

Double sections		
Tanderil tube feeding	$\bar{d} = -0.35$	S.E. = 0.28
	S.D. = 0.89	$n = 10$
Controls tube feeding	$\bar{d} = 0.01$	S.E. = 0.27
	S.D. = 1.40	$n = 26$
Triple sections		
Tanderil tube feeding		S.E. = 0.36
	S.D. = 1.13	$n = 10$

Personal factors. Comparison of results obtained by two examiners who estimated the amount new-formed bone independently of one another (performed on control material)

Person 1 $n = 33$	$M = 18.18$	S.E. = 1.96
Person 2 $n = 31$	$M = 17.79$	S.E. = 2.58
Paired differences for persons 1 and 2	$Md = 0.56$	S.E. = 1.66
	S.D. = 9.23	$n = 31$

RESULTS

The results are summarized in Tables 2 and 3. Immobilization of one of the legs in plaster (group A) produced no divergent periosteal bone formation compared with that in the non-immobilized leg. The short immobilization in plaster (14 days) thus had no effect on normal periosteal bone formation.

On immobilization of the leg from which marrow had been removed (group B) there was a collateral periosteal bone reaction with increased bone formation. The mean difference between the operated bone and the non-operated bone was 16.6 surface units, which did not differ from that (18.2 units) in a marrow evacuation control series in which the leg was not immobilized (see previous paper). See also the significance Table 3.

NaCl given by feeding tube to animals in which marrow had been evacuated (group C) diminished the periosteal reaction compared with the control series. The mean difference between the operated and the unoperated side was 5.9 units, a value that differed significantly from that in the control material.

Tanderil by feeding tube (group D) gave a mean difference of 9.3 units between the operated and the unoperated side. Compared with the group of animals given NaCl by feeding tube, there was no significant increase. Compared with the original control material (i.e. without placebo tube-feeding) the mean difference was significantly decreased.

Tube feeding with Difhydan (group E) gave a small mean difference (5.5 units) a result equal to that obtained with tube feeding of placebo (group C). The value suggests that Difhydan in our experimental design does not affect periosteal bone formation.

Tanderil given to operated animals subcutaneously (group F) produced a mean difference of 9.4 units. This value was significantly decreased, compared with that in the original control material.

Tanderil as injection requires a solvent whose chemical composition is I. triethylglycole 7% + II. ethylglycole-mono-ethyl ester 13% + H_2O.

The solvent given subcutaneously as placebo (group C) produced a mean difference of 9.5 units. This value is exactly the same as that obtained in the group that received Tanderil (+ solvent) subcutaneously (group F).

Table II. *Results*

Parameter	Mean difference (op.–non-op.)	S.E.	No. of animals	
Control material	18.18	1.96	33	
Plaster without op. (A)	0.09	0.20	18	
Plaster with op. (B)	16.63	2.08	20	
Controls tube fed with NaCl (C)	5.86	1.39	26	Double sections $n = 26$
Tanderil by feeding tube (D)	9.31	1.56	20	Double sections $n = 10$ Triple sections $n = 10$
Difhydan by feeding tube (E)	5.46	1.21	20	
Tanderil by injection (F)	9.38	3.59	17	
Controls Solvent for Tanderil inj. (G)	9.48	3.11	18	
Anabolic steroid (H)	12.40	2.42	19	

Table III. *Significance table*

	Control material $M=18.18$ S.E. = 1.96	A Plaster without op. $M=0.09$ S.E. = 0.20	B Plaster with op. $M=16.63$ S.E. = 2.08	C Tube fed NaCl $M=5.86$ S.E. = 1.39	D Tanderil sond-feeding $M=9.31$ S.E. = 1.21	E Difhydan sond-feeding $M=5.46$ S.E. = 1.21	F Tanderil injection $M=9.38$ S.E. = 3.59	G Solvent for Tanderil injection $M=9.48$ S.E. = 3.11	H Anabolic steroid $M=12.40$ S.E. = 2.42
Control material		***	N.S.	***	***	***	*	*	N.S.
Plaster without op.			***	***	***	***	*	*	***
Plaster with op.				***	***	*	N.S.	N.S.	N.S.
Tube feeding NaCl					N.S.	N.S.	N.S.	N.S.	*
Tanderil by feeding tube						*	N.S.	N.S.	N.S.
Difhydan by feeding tube							N.S.	N.S.	*
Tanderil by injection								N.S.	N.S.
Solvent for Tanderil injection									N.S.
Anabolic steroid									

* $0.01 < P < 0.05$. ** $0.001 < P < 0.01$. *** $P < 0.001$. N.S. = not significant.

Anadur given as an injection 5 days before the operation (group H) produced a mean difference of 12.6 units.

DISCUSSION

The purpose of the present investigation was to elucidate the effect, if any, of different factors on periosteal bone formation. The normal formation of periosteal bone may conceivably be influenced by such general factors as nutrition and the activity of the individual. It may also be assumed that the growth of the bone probably occurs in periods dependent on hitherto obscure factors. Standardized evacuation of marrow results in a periosteal growth due to a collateral reaction in the periosteum. This acute tissue proliferation runs a course that is, according to earlier investigations, statistically well defined. Obviously, the more standardised the experiments are the greater will be the reliability of the results. The purpose of this investigation was to find out whether various external circumstances, as well as drugs, influence the periosteal bone formation uniformly.

The administration of drugs to animals naturally means that one disturbs the animals' environments. However, we thought it reasonable to assume that a subcutaneous injection *per se* would not produce any notable change and therefore we did not check this point. But every time an animal is tube fed, it means a trauma. We therefore thought it desirable to test this by a blind test. The results of tube feeding alone suggest that such feeding causes a substantial reduction of bone formation, probably owing to a stress reaction. Tube feeding as a method of administration in experiments with various drugs therefore probably make it more difficult to evaluate the results obtained.

The change produced by immobilization in plaster for 2 weeks does not seem to influence periosteal bone formation to any notable extent. It is, however, known that immobilization in plaster *per se* produces a considerable change in the circulation of the blood in both the soft tissues surrounding the bone and in the actual bone,

so that one might expect certain changes in periosteal bone formation. The absence of this reaction should to a certain extent argue against a change in the blood flow—stasis—initiating the periosteal bone formation after evacuation of marrow.

The effect of the active component in Tanderil on periosteal bone formation is very difficult to assess. Judging from the results obtained with tube feeding of placebo, the active component of Tanderil may have a favourable effect on periosteal bone formation. One might wonder whether this effect is indirect via a decreased inflammatory edema in the limb after traumatization of the marrow, or whether it is a more direct effect. Tanderil given subcutaneously, like the solvent alone given subcutaneously, produces exactly the same periosteal bone formation. This may indicate that the injected substance has in some way interfered with the tissue proliferation in a toxic way.

The results of the experiments with Difhydan indicate that this preparation has neither a stimulating nor an inhibitory effect on periosteal bone formation in the experimental design used.

Administration of Anadur gives a difference of 12.4 units. The value is not significantly different from that in the control material. One would expect a stronger periosteal bone formation reaction. The cause of the low value may be that the anabolic effect on bone formation has not had time to develop in the period covered by the experiment. Also, factors unknown to us may have affected the results.

COMMENTS

The results may seem less uniform than expected. This might lead to a weakening of one's confidence in the experimental design. But, then again, a wide variation must be expected in the analysis of such a complex mechanism as tissue proliferation, in this case periosteal bone formation. This formation is certainly influenced by many still-unknown factors among which the general condition, treatment and care of the animals under the experimental period seems to be of utmost importance. The error of the method in the individual animal showed good reproducibility as did the study of the human error in the reading of the results. This is a good basis for further evaluation of unknown factors that might influence the results of periosteal bone formation in experiments using marrow evacuation.

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