JUKKA INKOVAARA
Associate Teacher of Medicine
Department of Clinical Sciences,
University of Tampere, Teiskontie
35, SF-33520 Tampere 52,
Finland
GUIDO GOTHONI
Medical Director
Research Laboratories of Medica
Pharmaceutical Company Ltd,
POB 326, SF-00101 Helsinki 10,
Finland
RAIJA HALTTULA
Registered Nurse in Koukkuniemi
Municipal Home
RAUNO HEIKINHEIMO
Chief Physician of Geriatric
Department
Tampere City Hospital,
Parantolankatu 6, SF-33500
Tampere 50, Finland
OLAVI TOKOLA
Assistant Medical Director
Research Laboratories of Medica
Pharmaceutical Company Ltd

Age and Ageing (1983) *12*, 124–130

CALCIUM, VITAMIN D AND ANABOLIC STEROID IN TREATMENT OF AGED BONES: DOUBLE-BLIND PLACEBO-CONTROLLED LONG-TERM CLINICAL TRIAL

Summary

In a double-blind trial, 327 patients (57 men) over 65 (mean age 79.5) years received all possible combinations of calcium carbonate 3 g, vitamin D_3 1000 iu, methandienone 2.5 mg and/or placebos daily for 9 months. The higher incidence of bone fractures in the placebo group was not significant. Serum calcium, phosphorus, creatinine, aspartate aminotransferase and alkaline phosphatase were followed: the greatest changes occurred with methandienone, which thus reduced osteoporotic activity and increased the muscular mass most effectively; calcium carbonate had the poorest effect. Surprisingly, coronary mortality was higher among those taking all three active substances. With two treatments the increase was not significant, but when both the groups receiving a combination of any two of the treatments were compared with those taking only one or neither of these two treatments, a significant increase in coronary deaths was seen, most significant ($P < 0.001$) in those receiving vitamin D_3 and methandienone.

Introduction

One reason for the increased disposition to bone fractures of elderly people is weakening of the bones. The most common and important bone-weakening disease is osteoporosis, in addition to which osteomalacia may sometimes occur, either alone or with the former (Aaron et al. 1974).

A number of substances, such as calcium, oestrogens, anabolic steroids, vitamin D, fluoride and calcitonin have been tried in the treatment of osteoporosis. Calcium has given both positive (Nordin et al. 1975, Spencer et al. 1976) and negative (Smith et al. 1975) results; as also has vitamin D (Nordin et al. 1980, Johnson et al. 1980), and a positive result (Riggs et al. 1976) for combined treatment with calcium and vitamin D has been reported. Anabolic steroids have been found to raise total body calcium (Chesnut et al. 1977), but in elderly people their effect is usually only anticatabolic (Laitinen 1978). As none of these treatments has clearly proved effective, we found that there was cause for further study. The purpose of our study was to clarify the effects of calcium, vitamin D and anabolic steroids, alone or in combination, in long-term treatment and prevention of osteoporosis in a large patient material.

Patients and Methods

The trial was performed with 327 residents (57 men) of Koukkuniemi municipal home for the aged, in Tampere city. The age range was 65–97 (mean age ± s.d. was 79.5 ± 7.1) years. Subjects

with functional disorders of the kidneys (serum creatinine > 150 mmol/l) or liver (serum aspartate aminotransferase > 40 iu/l or serum alkaline phosphate > 280 iu/l), kidney stones, or hypercalcaemia (serum calcium > 2.80 mmol/l) were excluded from the trial. Suspected osteoporosis at the start of the trial was not a cause for exclusion.

Patients were divided into eight groups according to year and month of birth. Four groups received calcium carbonate (Ca) 3 g daily (= 1.2 g of calcium), the other four the corresponding placebo. Vitamin D_3 (D_3) 1000 iu daily and placebo were given to four, and methandienone (M) 2.5 mg daily and placebo to four groups each. Thus, each patient received three preparations of which none, one, two, or all contained one of the active drugs. The placebos were identical with the corresponding active drugs as regards the composition of inert constituents. Cholecalciferol in D_3 pills was substituted by soya oil, methandienone in M tablets by lactose, and calcium carbonate in Ca mixture by microcrystalline cellulose in the placebos.

A double-blind trial technique was used. The code was known only to the drug manufacturer (Medica Pharmaceutical Company Ltd.). It was kept in a sealed envelope in the dispensary of the institution while the trial was in progress and was not opened until the results had been analysed. Any other medication that the patients might be receiving was continued as before.

Table I. Number of patients, sex and age, and primary diagnoses at the start of the trial in the different treatment groups

Treatment group	*Placebo*	*Ca*	*D_3*	*M*	*Ca+D_3*	*Ca+M*	*D_3+M*	*Ca+D_3+M*	*Total*
Number	42	42	45	26	46	36	43	47	327
Male/female	9/33	6/36	7/38	9/17	10/36	7/29	5/38	4/43	57/270
Age, years (mean ± s.d.)	79.4 ±6.1	80.8 ±6.4	79.8 ±5.8	79.8 ±5.6	78.7 ±7.9	79.0 ±6.4	79.3 ±6.7	80.3 ±6.9	79.5 ±7.1
Non-cardiovascular	18	20	18	8	11	8	10	14	107
Cerebrovascular	14	11	15	10	19	16	18	15	118
Peripheral vascular	1	4	2	—	2	2	3	2	16
Cardiac (non-coronary)	6	6	9	6	10	9	9	13	68
Cardiac (coronary)	3	1	1	2	4	1	3	3	18

Number of patients, sex distribution, mean age and primary diagnoses in each group are presented in Table I.

The trial started on 1st April 1978. Patients were followed for 1 year, treatment was given for the first 9 months. At the start and end of treatment patients were weighed and measured. Serum calcium, phosphate, creatinine, transaminase and alkaline phosphatase were determined at the start and 2, 9 and 12 months later. The subjective condition was evaluated on a four-grade scale where the highest grade was 'feeling well' and the lowest 'not feeling well'; symptoms were not specified. Other data recorded were: patients interrupting, reasons for interruption, bone fractures, removals to hospital and deaths.

Serum-calcium was determined by atomic absorption spectrometry (Cali et al. 1973), serum-phosphate by a modification of the method of Fiske and Subbarow (1925), serum-creatinine by absorption photometry (Clark & Thompson 1949), serum-aspartate aminotransferase (ASAT) and alkaline phosphatase (AFOS) by a kinetic absorption photometric method (Committee on Enzymes 1974).

From each group 10 patients were selected by lots for radiographic examination of the thoracic and lumbar portions of the spinal column and the wrist bones at the start and end of treatment.

For statistical analysis the chi-squared test and linear correlation coefficient were used.

Table II. Starting values and changes in laboratory parameters in the different treatment groups

	Placebo		*Ca*		*D_3*		*M*		*Ca+D_3*		*Ca+M*		*D_3+M*		*Ca+D_3+M*	
	Mean	±*s.d.*	*Mean*	±*s.d.*	*Mean*	±*s.d.*	*Mean*	±*s.d.*	*Mean*	±*s.d.*	*Mean*	±*s.d.*	*Mean*	±*s.d.*	*Mean*	±*s.d.*
Initially																
Ca	2.41	0.29	2.27	0.29	2.40	0.27	2.35	0.22	2.41	0.30	2.29	0.27	2.35	0.28	2.27	0.24
P	0.90	0.21	0.93	0.19	0.95	0.19	0.22	0.26	0.93	0.24	0.98	0.20	0.97	0.32	0.98	0.23
Creat	97.92	18.99	90.72	16.81	93.05	15.10	102.12	22.02	95.27	17.20	92.90	16.47	99.18	18.09	96.97	17.67
ASAT	20.35	4.95	19.64	4.51	18.95	3.86	18.50	4.44	18.70	4.31	18.03	4.30	20.18	5.63	20.31	4.21
AFOS	184.25	56.10	181.56	45.67	177.25	41.88	173.71	41.10	177.82	56.94	189.97	51.04	186.24	49.78	190.08	53.00
2 months																
Ca	−0.09		+0.02		−0.10*		−0.10		−0.08		−0.01		−0.05		+0.04	
P	+0.12**		+0.08*		+0.08**		+0.02		+0.10*		−0.05		+0.03		+0.01	
Creat	+4.97**		+6.33**		+5.17*		+12.30**		+7.50**		+13.00**		+12.08**		+16.67**	
ASAT	−0.53		−1.20		−0.63		+7.50**		+0.10		+11.04**		+8.88*		+6.51**	
AFOS	+21.15**		−3.03		−4.00		−37.95**		−11.37		−34.84**		−44.39**		−45.77**	
9 months																
Ca	+0.07		+0.18**		+0.08		+0.06		+0.11		+0.12		+0.21**		+0.23**	
P	+0.15**		+0.18**		+0.13**		+0.10		+0.21**		+0.03		+0.11		+0.05	
Creat	+4.39		+4.78		+6.94*		+11.86**		+5.13*		+21.25**		+12.10**		+14.54**	
ASAT	+0.16		−1.22		−0.64		+3.98		−0.52		+15.57**		+5.71*		+8.88*	
AFOS	+17.23		−6.82		−9.47		−22.34		−4.26		−32.81*		−57.67**		−58.75**	
12 months																
Ca	+0.09		+0.20**		+0.06		+0.09		+0.06		+0.22*		+0.22**		+0.26**	
P	+0.17**		+0.15**		+0.16**		+0.18**		+0.22**		+0.06		+0.15*		+0.07	
Creat	−0.30		+5.00		+4.09		+0.00		+1.00		+4.29		+2.15		+3.08	
ASAT	−1.10		+0.62		+0.87		+2.21		+3.57		+2.64*		+4.95*		+1.79*	
AFOS	+24.17**		−18.62		+11.53		+18.71		+17.63		+9.21		−0.90		−9.58	

* $P<0.05$. ** $P<0.01$.

Results

Starting values and changes in laboratory parameters during treatment and follow-up are given in Table II. Table III shows changes in laboratory values of the active treatment groups compared with those of the placebo group.

Ca reduced AFOS, which was seen particularly after 2 months' treatment. When Ca was combined with M, a reduction in serum-phosphate was also seen after 2 months, and the creatinine- and ASAT-increasing effects of M were potentiated.

Table III. Changes in laboratory values of the active drug groups as compared with the placebo group

	Months	*Ca*	*P*	*Creat*	*ASAT*	*AFOS*
Ca	2	—	—	—	—	↓↓
	9	—	—	—	—	↓
	12	—	—	—	—	—
D_3	2	—	—	—	—	↓↓
	9	—	—	—	—	↓
	12	—	—	—	—	↓↓
M	2	—	—	↑	↑↑	↓↓
	9	—	—	—	—	↓
	12	—	—	—	↑	—
$Ca+D_3$	2	—	—	—	—	↓↓
	9	—	—	—	—	—
	12	—	—	—	—	—
Ca+M	2	—	↓↓	↑	↑↑	↓↓
	9	—	—	↑↑	↑↑	↓↓
	12	—	—	—	↑↑	—
D_3+M	2	—	—	↑	↑	↓↓
	9	—	—	↑	—	↓↓
	12	—	—	—	↑↑	—
$Ca+D_3+M$	2	↑	—	↑	↑↑	↓↓
	9	↑	—	↑↑	↑	↓↓
	12	↑	—	—	↑	↓

— = not significant; ↑ or ↓ = $P<0.05$; ↑↑ or ↓↓ = $P<0.01$.

Likewise, D_3 reduced AFOS even when used alone. Mean serum-calcium was not increased by D_3, but three patients developed a transient hypercalcaemia (calcium > 2.80 mmol/l) during the 9 months of treatment; one of them received D_3, one D_3+M and one D_3+M+Ca.

M caused the most pronounced changes: it raised serum-creatinine and ASAT and reduced AFOS both alone and in combinations. At the end of the treatment (9 months), ASAT was above the reference value 40 iu in nine patients, but at the end of the follow-up period (12 months) in only one. Creatinine exceeded 150 mmol/l in six patients at 9 months and in two at 12 months.

Table IV shows that the weight and height of patients did not change significantly in any group. Bone fractures were more frequent in the placebo group, but the difference was not significant.

Table IV. Changes in weight and height during the 9-month treatment period: bone fractures, interruptions of treatment, and deaths during the 12-month observation period

	Placebo	*Ca*	*D_3*	*M*	*Ca+D_3*	*Ca+M*	*D_3+M*	*Ca+D_3+M*	*Total*
Weight, kg	−0.10	−0.45	−0.06	−1.60	−0.65	−0.36	−1.23	−0.87	
Height, cm	−0.40	−0.75	−0.70	−0.80	−0.46	+0.12	−0.66	−0.62	
Fractures	3	1	1	1	0	1	1	2	10
Interrupted (died)	14 (5)	11 (6)	13 (7)	14 (8)	16 (7)	25 (7)	24 (11)	23 (16)	140 (67)

Of the 140 interruptions, 113 were due to hospitalization, as is seen in Table V. Cerebrovascular disorder as the cause of hospitalization seems to be more frequent among patients who took D_3 (28/181) than among those who did not (14/146). Of those taking M + D_3, more patients (7/90) went into hospital with coronary disease than in the other groups (3/237). These differences, however, are not statistically significant.

More patients died suddenly of coronary disease and peripheral vascular diseases (Table VI) than went into hospital for them (Table V). Coronary disease was the only fatal cardiac disorder occurring.

Table V. Reasons for admission to hospital

	Placebo	*Ca*	*D_3*	*M*	*Ca+D_3*	*Ca+M*	*D_3+M*	*Ca+D_3+M*	*Total*
Non-cardiovascular	7	9	6	6	7	6	8	6	55
Cerebrovascular	3	2	8	3	7	6	7	6	42
Peripheral vascular	–	–	–	–	–	–	–	1	1
Cardiac (non-coronary)	2	–	–	2	–	1	–	–	5
Cardiac (coronary)	–	1	–	1	1	–	3	4	10
	12	13	14	12	15	13	18	17	113

Table VI. Mortality in the different treatment groups (men in parenthesis)

	Placebo	*Ca*	*D_3*	*M*	*Ca+D_3*	*Ca+M*	*D_3+M*	*Ca+D_3+M*	*Total*
Patients	42 (9)	42 (6)	45 (7)	26 (9)	46 (10)	36 (7)	43 (5)	47 (4)	327 (57)
Non-cardiovascular	2 (2)	1 (1)	2	4 (2)	2 (1)	1	1	2	25 (6)
Cerebrovascular	2	1 (1)	4	2	2	5	4	4 (1)	24 (2)
Peripheral vascular	–	1 (1)	1	1	–	–	2	–	5 (1)
Cardiac (coronary)	1	3 (1)	–	1 (1)	3 (1)	1	4	10 (1)	23 (4)
Total mortality	5 (2)	6 (4)	7	8 (3)	7 (2)	7	11	16 (2)	67 (13)

From Table VI it appears that the total mortality was highest ($P < 0.05$) in the group taking all three active preparations. The difference is seen principally in coronary mortality (10/47 against 13/280; $P < 0.001$); with regard to other causes of death the groups did not differ significantly. Also, for women only, the difference in coronary mortality was significant ($P < 0.001$).

Among patients taking two active preparations, the coronary mortality (8/125) was somewhat, though not significantly, higher than among those taking one or none (5/155). If, on the other hand, the relationship of each treatment combination (D_3+Ca; $M+Ca$ and D_3+M) with coronary mortality is studied, it is seen that the latter is higher for any combination ($P<0.01$; <0.05 and <0.001, respectively) than in the groups receiving only one or none of the particular preparations. The most significant increase was seen when D_3 and M were combined (the groups D_3+M and D_3+M+Ca); this applied both to the whole series and to the women.

For those treated with M, the total mortality (42/152) and coronary mortality (16/152) were higher ($P<0.01$) than for the others (25/175 and 7/175). For those taking D_3 or those taking Ca, this difference was not significant.

The subjective condition of patients was similar in all groups. Radiography at the start and end of treatment revealed no differences between groups with regard to measures of the cortical layer and medullary cavities of the wrist bones.

Discussion

The patients in this trial were grouped strictly according to time of birth; thus, the groups are not identical with regard to numbers of patients and sex distribution. This complicates the statistical analysis but does not affect the reliability of the results. Even the mean age in the groups varied somewhat, but not so much as to be responsible for the differences, e.g. in coronary deaths. Diseases were fairly evenly distributed over the groups at the start of the trial. Only in 18 of 327 patients was coronary disease the primary disorder. Because each treatment was given to four groups and each combination of two preparations to two groups, the morbidity and mortality in these combined groups were also studied.

M had the strongest effect on the measured laboratory parameters. The M-induced rise in creatinine was probably a sign of improved anabolism and increased muscular mass. A decrease in AFOS was observed in all the active treatment groups and was believed to come of a reduction of osteoblastic activity. Raised ASAT again was clearly related to the use of M. No great changes occurred in serum calcium and phosphate values. Serum-calcium rose in the three-treatment group and a decrease in serum-phosphate was seen at 2 months in the $Ca+M$ group. In an earlier study (Johnson et al, 1980), vitamin D 2000 iu daily inhibited a fall in serum-phosphate and caused hypercalcaemia. In the present study, D_3 1000 iu daily caused hypercalcaemia in three patients.

The incidence of bone fractures was low (10/327 in 12 months) in this study, and although they were more frequent in the placebo group, no significance could be established. In a study performed in 1971–72 at the same institution (Inkovaara et al. 1975), half of the patients received sodium monofluorophosphate 25 mg daily for 5 months and twice a week for an additional 3 months. During the 9-month observation period 14/237 patients in the active treatment group and 6/233 in the control group had bone fractures. When compared with these results, the preparations in the present study cannot be said to increase the incidence of bone fractures as does fluoride, but rather decrease it.

A quite surprising observation was the clear increase in coronary deaths—and consequently in total death rate—when all three active preparations or even two, particularly

M + D_3, were used. Vitamin D in long-term use has been suspected of causing cardiac infarction (Linden 1974). Testosterone is known to increase thrombocyte aggregation and perhaps other coronary risk factors (Johnson et al. 1975, 1977, Pilo et al. 1981). Aloia et al. (1981) found three cardiovascular (two coronary) deaths in a series receiving anabolic steroid and none in the controls. In the present series neither M nor D_3 alone, but only the two together, seemed to increase coronary morbidity and mortality and possibly only in women. Thus, simultaneous use of these two drugs should apparently be avoided.

References

Aaron, J. E., Gallagher, J. C., Anderson, J., Stasiak, L., Longton, E. B., Nordin, B. E. C. & Nicholson, M. (1974) Frequency of osteomalacia and osteoporosis in fractures of the proximal femur. *Lancet i*, 229–33.

Aloia, J. F., Kapoor, A., Vaswani, A. & Cohn, S. H. (1981) Changes in body composition following therapy for osteoporosis with methandrostenolone. *Metabolism 30*, 1076–9.

Cali, J. P., Bowers, G. N., Jr, & Young, D. S. (1973) A referee method for the determination of total calcium in serum. *Clin. Chem. 19*, 1208–13.

Chesnut, C. H., Nelp, W. B., Baylink, D. J. & Denney, J. D. (1977) Effect of methandrostenolone on postmenopausal bone wasting as assessed by changes in total bone mineral mass. *Metabolism 26*, 267–77.

Clark, L. C., Jr, & Thompson, H. L. (1949) Determination of creatine and creatinine in urine. *Anal. Chem. 21*, 1218–21.

Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. (1974) Recommended methods for the determination of four enzymes in blood. *Scand. J. Clin. Lab. Invest. 33*, 291–306.

Fiske, C. H. & Subbarow, Y. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem. 66*, 375–400.

Inkovaara, J., Heikinheimo, R., Järvinen, K., Kasurinen, U., Hanhijärvi, H. & Iisalo, E. (1975) Prophylactic fluoride treatment and aged bones. *Br. Med. J. ii*, 73–4.

Johnson, K. R., Jobber, J. & Stonawski, B. J. (1980) Prophylactic vitamin D in the elderly. *Age Ageing 9*, 121–7.

Johnson, M., Ramey, E. & Ramwell, P. W. (1975) Sex and age differences in human platelet aggregation. *Nature 253*, 355–7.

Johnson, M., Ramey, E. & Ramwell, P. W. (1977) Androgen-mediated sensitivity in platelet aggregation. *Am. J. Physiol. 232*, H381–5.

Laitinen, O. (1978) Osteoporosis. *Public Health Rev. 7*, 169–95.

Linden, V. (1974) Vitamin D and myocardial infarction. *Br. Med. J. 2*, 647–50.

Nordin, B. E. C., Horsman, A., Crilly, R. G., Marshall, D. H. & Simpson, M. (1980) Treatment of spinal osteoporosis in postmenopausal women. *Br. Med. J. 280*, 451–4.

Nordin, B. E. C., Horsman, A. & Gallagher, J. C. (1975) Effect of various therapies on bone loss in women. In: *Calcium Metabolism, Bone and Metabolic Bone Diseases* Ed.: F. Kuhlencordt and H-P. Kruse. Berlin: Springer-Verlag, pp. 233–42.

Pilo, R., Aharony, D. & Raz, A. (1981) Testosterone potentiation of ionophore and ADP induced platelet aggregation. *Thromb. Haemostas. 46*, 538–42.

Riggs, B. L., Jowsey, J., Kelly, P. J., Hoffman, D. L. & Arnaud, C. D. (1976) Effects of oral therapy with calcium and vitamin D in primary osteoporosis. *J. Clin. Endocrinol. Metab. 42*, 1139–44.

Smith, D. A., Anderson, J. J. B., Aitken, J. M. & Shimmins, J. (1975) The effects of calcium supplementation of the diet on bone mass in women. In: *Calcium Metabolism, Bone and Metabolic Bone Diseases* Ed.: F. Kuhlencordt and H-P. Kruse. Berlin: Springer-Verlag. pp. 278–82.

Spencer, H., Kramer, L., Osis, D. & Norris, C. (1976) Effect of conventional and newer forms of treatment on calcium absorption in osteoporosis. *Israel J. Med. Sci. 12*, 638–51.