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Use of biochemical markers to monitor changes in bone turnover in cynomolgus monkeys

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The ovariectomized old cynomolgus monkey is a recognized model of human osteoporosis, and the same species can be used for the assessment of the efficacy and potential toxicity of agents intended to prevent or treat osteoporosis. Several assays have been developed that can measure the same biochemical markers of bone turnover as are used in human patients for the diagnosis and treatment follow-up of bone-related diseases, including osteoporosis. The aim of the present study was to describe the results obtained with these assays in normal control monkeys, their variations with age and sex, and their sensitivity in monitoring the bone turnover induced by ovariectomy in old skeletally mature cynomolgus monkeys. Seven old cynomolgus monkeys were bilaterally ovariectomized and 13 age-matched monkeys were sham-operated. Bone mineral density and biochemical markers were measured before and at regular intervals after surgery for up to 20 months. Total alkaline phosphatase (total ALP), bone-specific alkaline phosphatase isoenzyme (bone ALP) and osteocalcin (OC) were highly correlated to the decrease in bone mineral density (BMD) induced by ovariectomy. Deoxypyridinoline (DPD) measured by enzyme-linked immunoassay was insensitive to the bone resorption induced by ovariectomy, but cross-linked N-telopeptide (NTX-I) was higher in ovariectomized monkeys than in control monkeys. These results demonstrate that reliable biochemical parameters are available to adequately monitor and provide insight into osteoclastic bone resorption and osteoblastic bone formation, the two components of bone turnover in this animal model, and can thus be used to assess the efficacy and toxicity of potential therapeutic agents.

Keywords: monkey, osteoporosis, biochemical markers, bone mineral density, ovariectomy.

Introduction

The osteopenic, ovariectomized, old cynomolgus monkey is a recognized model of human osteoporosis (Brommage 2001) and has been used to investigate the effects of anti-resorptive and anabolic potential therapeutic agents (Brommage *et al*. 1999b, Jerome *et al*. 1999). The use of the same species for preclinical safety assessment in a non-rodent species gives the unique opportunity to compare the potential efficacy and adverse event profile of potential therapeutic agents. As in humans, the trabecular and cortical parts of the bones of cynomolgus monkeys undergo continuous bone turnover through sequential resorption and formation

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Table 1. Validated assays for biochemical parameters potentially used as markers of bone turnover in laboratory animals.

Parameters	Sample	Rat	Cynomolgus monkey	Dog
Bone formation				
Bone ALP	Serum	Ez	EIA	EIA
OC	Serum	RIA	EIA	RIA
PICP	Serum	–	EIA	–
Bone resorption				
Pyridinoline	Serum	–	EIA	EIA
ICTP	Serum	RIA	RIA	RIA
CTX-I	Serum/urine	–	EIA	–
Free DPD	Urine	EIA	EIA	EIA
NTX-I	Urine	EIA	EIA	–

Bone ALP, bone-specific alkaline phosphatase; CTX-I, C-telopeptide; DPD, deoxypyridinoline; ICTP, C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinases; NTX-I, N-telopeptide; OC, osteocalcin; PICP, procollagen type I C propeptide.

EIA, enzyme-linked immunoassay; Ez, enzymatic assay; RIA, radioimmunoassay; —, no assay available.

within bone remodelling units (Bouvier and Hylander 1996). Several tools are available to monitor the bone status of these animals, including the measurement of the bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) (Jerome *et al.* 1997b) or peripheral quantitative computed tomography (pQCT) (Hotchkiss 1999) and the histomorphometric analysis of undecalcified bone biopsy samples from the iliac crests and ribs (Goodwin and Jerome 1987). Blood and urine biochemical parameters can be measured rapidly, relatively inexpensively and repetitively and may provide a dynamic assessment of the skeleton (Calvo *et al.* 1996). Various assays are available to measure biochemical parameters that can be potentially used as markers of bone turnover in laboratory animals, especially in the cynomolgus monkeys, where specific enzyme-linked immunoassays (EIAs) have been recently developed and validated (table 1). The nomenclature of the bone biochemical markers currently available in humans has been recently summarized by Delmas (2001). The objective of the present paper is to list those markers that can currently be measured in animals, usually for the preclinical assessment of the efficacy and safety of agents intended to prevent or treat osteoporosis, their values in normal control cynomolgus monkeys, and their correlation with bone turnover in normal and ovariectomized old cynomolgus monkeys.

Materials and methods

Animals

The male and female cynomolgus monkeys of various ages that were used for this study came from Mauritius. Young animals, 2–3 years old, were purpose-bred and served as control animals in various toxicology studies. Old mature animals were originally obtained by capture, but they were kept in breeding gangs for several years before inclusion in the study and their age was estimated to be older than 10 years based on their history, body weight and dentition. All animal manipulations were performed under the guidelines of state laws, the French Ministry of Agriculture, and the institutional animal care and use committee.

Ovariectomy-induced osteopenia

Two groups of old female cynomolgus monkeys (mean weight 4 kg) were included in the study and randomly allocated to two groups. For the surgical procedures and BMD measurements, the animals were anaesthetized by an intramuscular injection of ketamine hydrochloride (Imalgène® 20 mg kg^{-1}, Mérial, Lyon, France) and xylazine hydrochloride (Rompun®, 0.3 ml/animal, Bayer Pharma, Puteaux, France). The animals of the first group were sham-operated (SHAM, $n = 13$) and those of the second group were ovariectomized (OVX, $n = 7$). All monkeys were then kept in individual cages for 20 months, and fed a diet containing 0.95% calcium, 0.84% phosphorus and 2000 IU kg^{1} vitamin D_3 throughout the study. The effectiveness of the ovariectomy procedure was assessed by an assay of serum oestradiol and by the systematic macroscopic research of ectopic residual ovarian tissue at necropsy.

The development of osteopenia was monitored by sequential measurements of BMD, which is considered to be a reliable marker of bone turnover. A QDR-2000 X-ray bone densitometer (Hologic, France) was used to measure the *in vivo* BMD of the lumbar vertebrae. The bone mineral content (BMC) and bone area of the lumbar L2–L5 vertebral block were measured from ventrodorsal projections using the 'spine' scan mode (space between lines set at 0.1003 cm, and space resolution set at 0.0965 cm). BMD was then calculated as the BMC divided by the projected bone area using the 'low density spine' scan option. The coefficient of variance of laboratory BMD measurements was 1.9% (Fisch and Forster 1998a). The measurements were performed twice at each time for each animal before surgery, and 3, 6, 9, 13, 16 and 20 months after surgery. The mean of the two measurements was calculated for each animal at each time-point.

Biochemical parameters measured for osteopenia development

Blood and urine were collected from all animals three times before surgery (over a 10 day period), and 3, 6, 9, 13, 16 and 20 months after surgery (twice on each occasion over a 5 day period) for the determination of serum and urinary biochemical markers. All blood samples were collected at the same time of day, during the morning, to avoid circadian variations in the parameters. The animals were deprived of food and water for an overnight period of at least 14 h before urine collection and blood sampling. Total alkaline phosphatase activity (total ALP) was measured in the serum by an enzymatic method (ALP, DGKC/30°C, Roche), creatinine was measured in the urine by Jaffé method (Roche). All other parameters were quantified by EIAs using standardized procedures similar to those initially described in humans (table 2) (Delmas *et al*. 1990, Robins *et al*. 1994). Osteocalcin (OC), procollagen I carboxy-terminal propeptide (PICP), deoxypyridinoline (DPD) and N-terminal cross-linking telopeptide of type I collagen (NTX-I) were measured by competitive immunoassays using murine monoclonal antibodies (Tracy *et al*. 1990, Hanson *et al*. 1992, Seyedin *et al*. 1993). Bone-specific alkaline phosphatase (bone ALP) was measured by a sandwich immunoassay using a murine monoclonal IgG antibody (Price 1993). At each time, and for each biochemical parameter, the mean of the two measures was calculated for each animal. The nomenclature and abbreviations used for bone markers are those recommended by the committee of scientific advisors of the international osteoporosis foundation (Delmas 2001).

Statistical analysis

All data are presented as the mean + SEM. They were tested for equality of variances.

Table 2. Enzyme-linked immunoassays used for the quantification of biochemical parameters in cynomolgus monkeys.

Parameters	Sample	Immunoassay used in cynomolgus monkeys
Bone formation		
Bone ALP	Serum	Alkaphase-B® (Quidel)
OC	Serum	NovoCalcin® (Quidel)
PICP	Serum	Prolagen-C® (Quidel)
Bone resorption		
Pyridinoline	Serum	Pyrilinks® (Quidel)
ICTP	Serum	RIA (Orion Diagnostica Pharmacia)
CTX-I	Serum/urine	CrossLaps® (Osteometer)
Free DPD	Urine	Pyrilinks-D® (Quidel)
NTX-I	Urine	Osteomark® (Ostex)

Bone ALP, bone-specific alkaline phospatase; CTX-I, C-telopeptide; DPD, deoxypyridinoline; ICTP, C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinases; NTX-I, N-telopeptide; OC, osteocalcin; PICP, procollagen type I C propeptide.

Longitudinal data (BMD, bone biomarkers) were analysed by repeated measures analysis of covariance (ANCOVA), with baseline data as the covariates and multiple post-baseline data as the repeated measures. If significant, *t*-tests were used to analyse the differences between OVX and SHAM animals. The group effect (OVX versus SHAM) was considered to be significant for P values < 0.05.

In old OVX and SHAM animals, linear least squares regression analyses were performed between total ALP and bone ALP, and total ALP and OC, using paired values for each animal at each sampling time. In addition, linear regression analysis was also performed between changes in total ALP, OC and BMD measured 6 months after surgery in these animals and expressed as a percentage of baseline (pre-surgery) values.

Results

Biochemical parameter values in normal cynomolgus monkeys

The collection of data obtained in normal Mauritius cynomolgus monkeys and summarized in table 3 was obtained using EIAs. Although enzymatic methods have previously been used for the quantification of some parameters (i.e. bone ALP, tartrate-resistant acid phosphatase), these assays were not reproducible and did not provide reliable results. Some of the assays were made available only recently and the database for these parameters in our laboratory was not large enough to allow valuable analysis.

Except for urinary NTX-I and ICTP, which were not statistically different between the two groups, all bone resorption (DPD, urinary CTX-I and serum CTX-I) and bone formation (bone ALP, OC, PICP) parameters were statistically higher in young male animals than in young female animals. In addition, except for DPD, all resorption (NTX-I) and formation (bone ALP, OC, PICP) markers were statistically higher in young than in old control female cynomolgus monkeys.

Osteopenia development after ovariectomy

The BMD of SHAM animals remained constant after surgery, whereas the BMD of OVX animals decreased after ovariectomy to values that were statistically

Table 3. Biochemical parameters potentially used as markers of bone turnover: mean ± SEM in control cynomolgus monkeys.

			Females	
Parameter	Sample	Young males (2–3 years)	Young (2–3 years)	Mature (> 10 years[a])
Bone formation				
Bone ALP ($IU\,l^{-1}$)	Serum	462 ± 19 ($n = 72$)	340 ± 14 ($n = 24$)	111 ± 6 ($n = 134$)
OC ($ng\,ml^{-1}$)	Serum	49 ± 2 ($n = 186$)	38 ± 1 ($n = 142$)	23 ± 1 ($n = 665$)
PICP ($ng\,ml^{-1}$)	Serum	373 ± 26 ($n = 81$)	285 ± 38 ($n = 26$)	115 ± 4 ($n = 564$)
Bone resorption				
Pyridinoline ($nmol\,l^{-1}$)	Serum	2.55 ± 0.01 ($n = 48$)	–	–
ICTP ($mg\,l^{-1}$)	Serum	23.0 ± 5.0 ($n = 20$)	24.1 ± 1.0 ($n = 20$)	–
CTX-I ($nM\,l^{-1}$)	Serum	13.1 ± 0.8 ($n = 30$)	9.8 ± 0.6 ($n = 28$)	–
CTX-I ($nM\,l^{-1}$)	Urine	2045 ± 998 ($n = 10$)	1381 ± 153 ($n = 10$)	–
DPD ($nM\,mM^{-1}$)	Urine	17.2 ± 0.6 ($n = 181$)	12.9 ± 0.3 ($n = 823$)	
NTX-I ($nM\ ECO\,mM^{-1}$)	Urine	571 ± 54 ($n = 20$)	622 ± 3 ($n = 20$)	116 ± 7 ($n = 134$)

Bone ALP, bone-specific alkaline phosphatase; CTX-I, C-telopeptide; DPD, deoxypyridinoline; ICTP, C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinases; NTX-I, N-telopeptide; OC, osteocalcin; PICP, procollagen type I C propeptide; –, no values available.

[a] Estimated age.

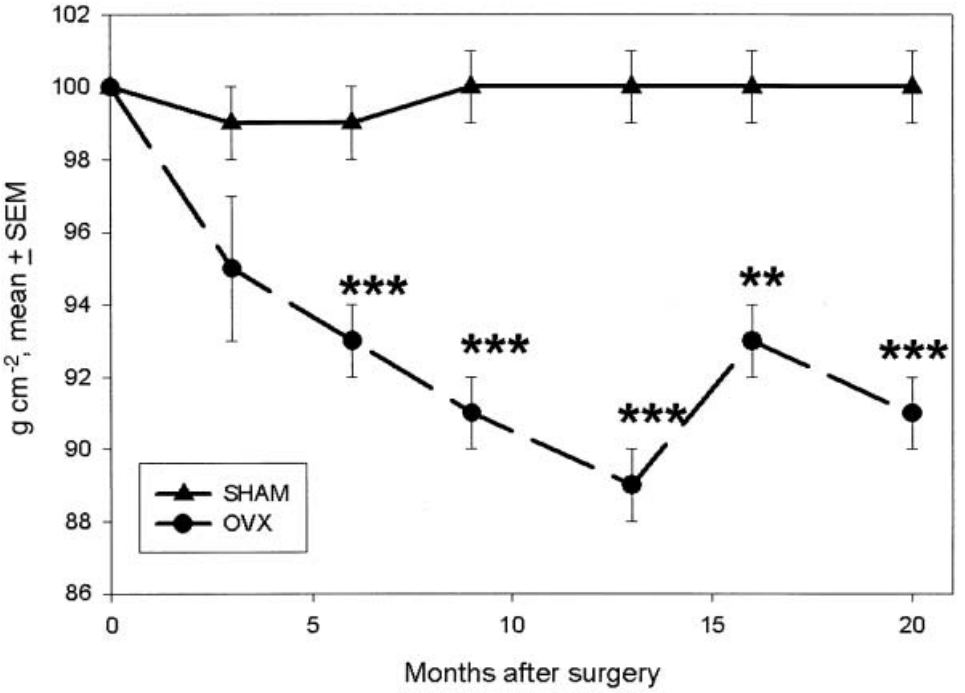

Figure 1. BMD evolution in SHAM and OVX old female cynomolgus monkeys. $^{**}p < 0.01$; $^{***}p < 0.001$.

significantly lower than pre-ovariectomy and SHAM values from 6 months after surgery. The decrease in BMD in ovariectomized animals was biphasic, with a rapid onset reaching a nadir 9 months after surgery and then remaining approximately stable at 10% lower than SHAM or pre-ovariectomy BMD levels (figure 1). The variations in the mean BMD observed at 13 and 20 months in this OVX group are mainly due to erratic values obtained in two animals.

Biochemical parameters during osteopenia development after ovariectomy

Although the pre-surgery values of DPD were lower in SHAM than in OVX animals, no statistically significant difference was observed for this parameter between OVX and SHAM animals at any time after surgery (figure 2).

NTX-I to creatinine urine ratios in SHAM animals did not change between the two sampling times. The levels measured in ovariectomized animals were significantly higher by approximately two-fold than those measured in SHAM animals at both 16 and 20 months after surgery (figure 3).

Total ALP serum activity remained stable in SHAM monkeys throughout the study. In OVX monkeys, this parameter increased dramatically by 3 months after surgery to values statistically higher than SHAM values, and then remained stable thereafter at the same high levels (figure 4).

OC serum levels remained stable in SHAM monkeys. In OVX animals, this parameter increased sharply by 3 months after surgery, remained at the same high level at 6 months after surgery, then slightly decreased by 9 months and remained stable at this elevated level thereafter (figure 5).

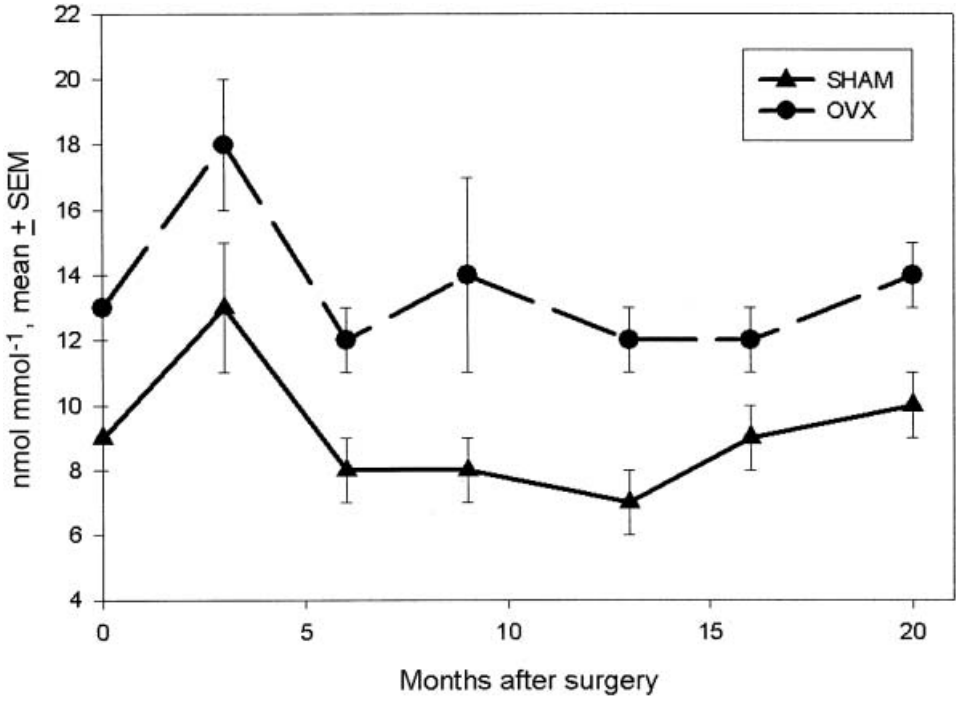

Figure 2. Urinary free DPD to creatinine ratio in SHAM and OVX old female cynomolgus monkeys.

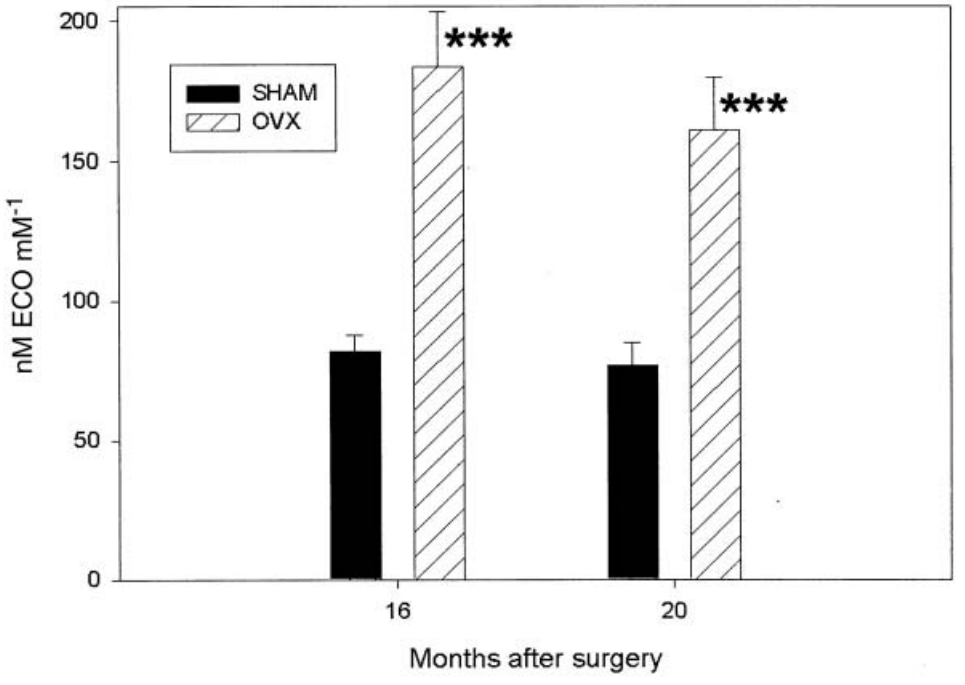

Figure 3. Urinary NTX-I in SHAM and OVX old cynomolgus monkeys 16 and 20 months after surgery. ***$p < 0.001$.

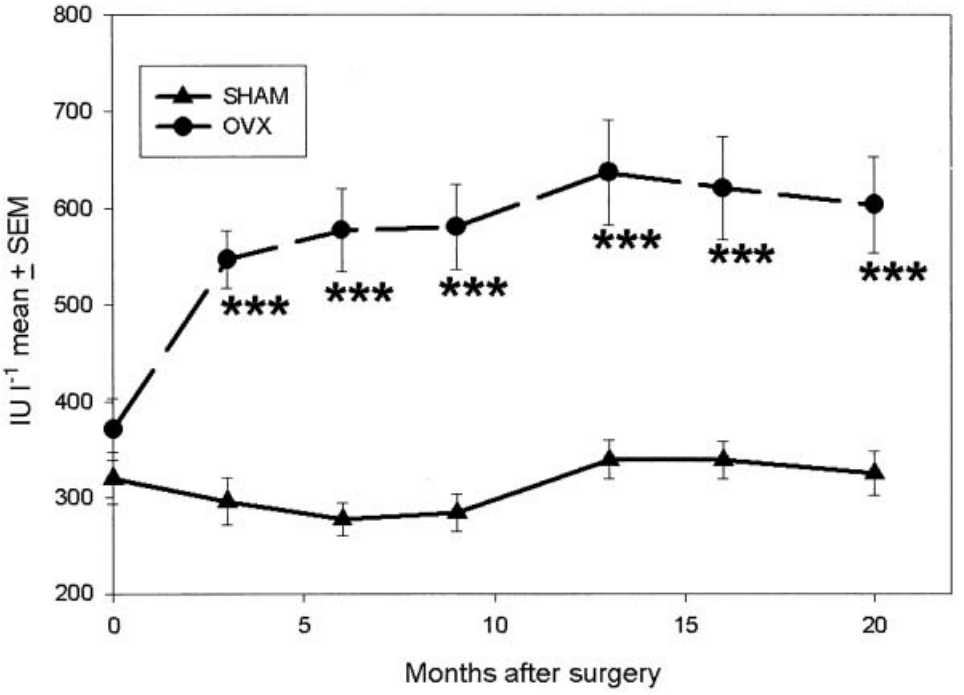

Figure 4. Serum total ALP activity in SHAM and OVX old female cynomolgus monkeys. ***$p < 0.001$.

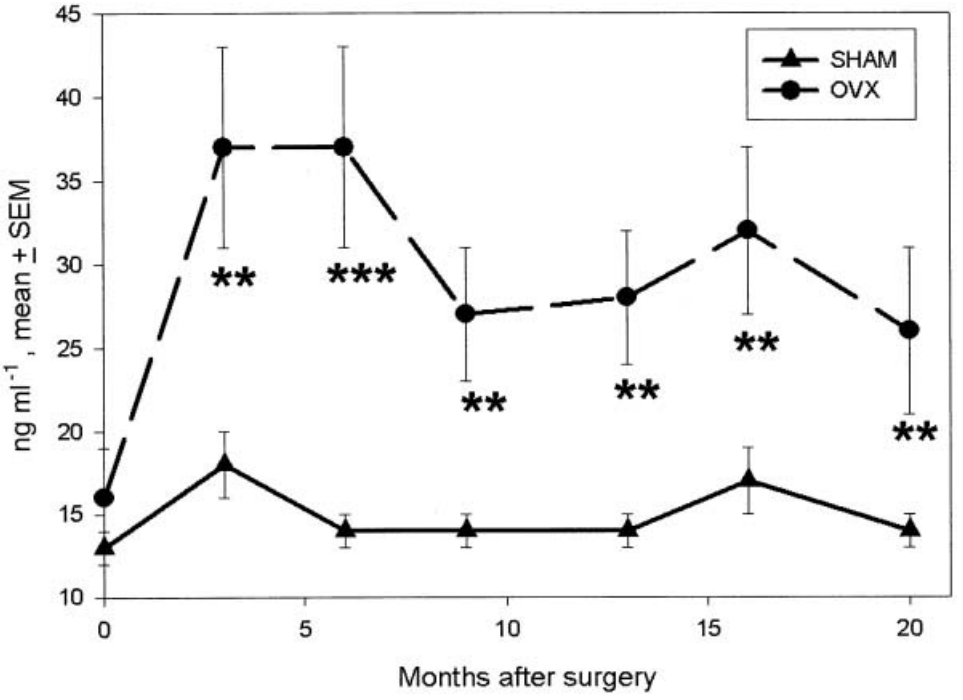

Figure 5. Serum OC in SHAM and OVX old female cynomolgus monkeys. **$p < 0.01$; ***$p < 0.001$.

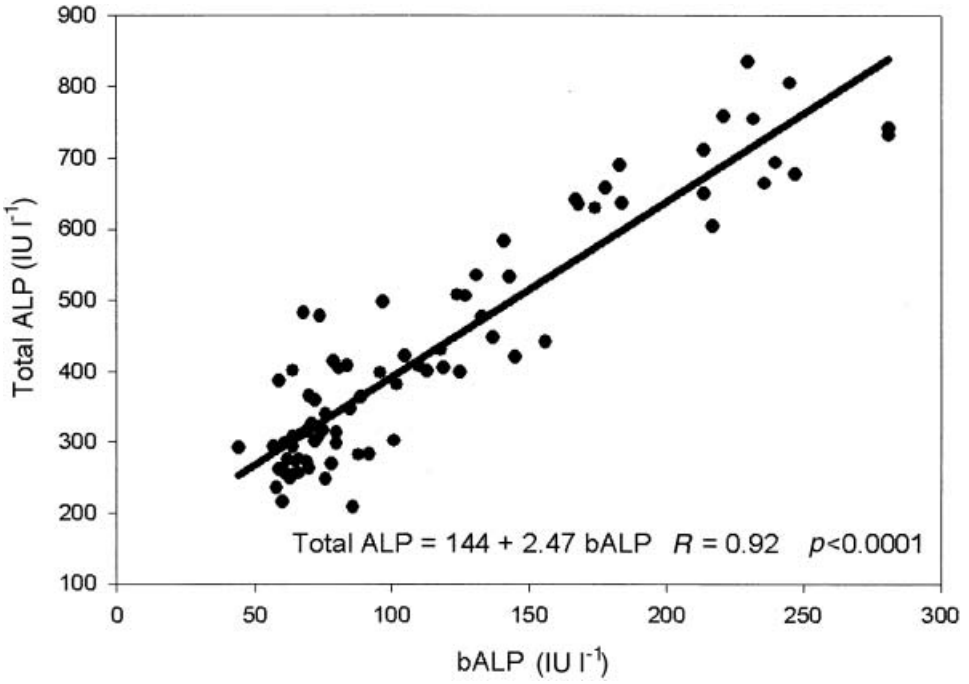

Figure 6. Correlation between total ALP and bone ALP (bALP) activities in SHAM and OVX old female cynomolgus monkeys.

PICP levels were not statistically different at any time between OVX and SHAM animals. No change in PICP levels was observed in OVX animals after surgery.

Total ALP serum activity, measured by enzymatic assay, was highly linearly correlated ($R = 0.92$, $p < 0.0001$) with bone ALP serum activity measured by specific EIA in SHAM and OVX old female cynomolgus monkeys (figure 6).

Total ALP serum activity was also linearly correlated with serum OC (figure 7). However, the residual was high ($R = 0.66$), reflecting dispersion within the high ranges.

When all parameters were expressed as a percentage of the pre-surgery value for each animal, both OC and ALP changes at 6 months were statistically ($p < 0.001$) inversely correlated to the BMD change at the same time ($R = 0.81$ and 0.74, respectively) (figures 8 and 9).

Discussion

Biochemical parameter values in normal cynomolgus monkeys

Although the circadian variations in most of the biochemical markers described in humans have not been studied in cynomolgus monkeys, all samples were taken approximately at the same period of the day to avoid any variability linked to this possible bias. The high intra- (day-to-day) and inter-individual variability that was observed for almost all parameters in cynomolgus monkeys has previously been described in humans (Garnero *et al.* 1994, Panteghini *et al.*

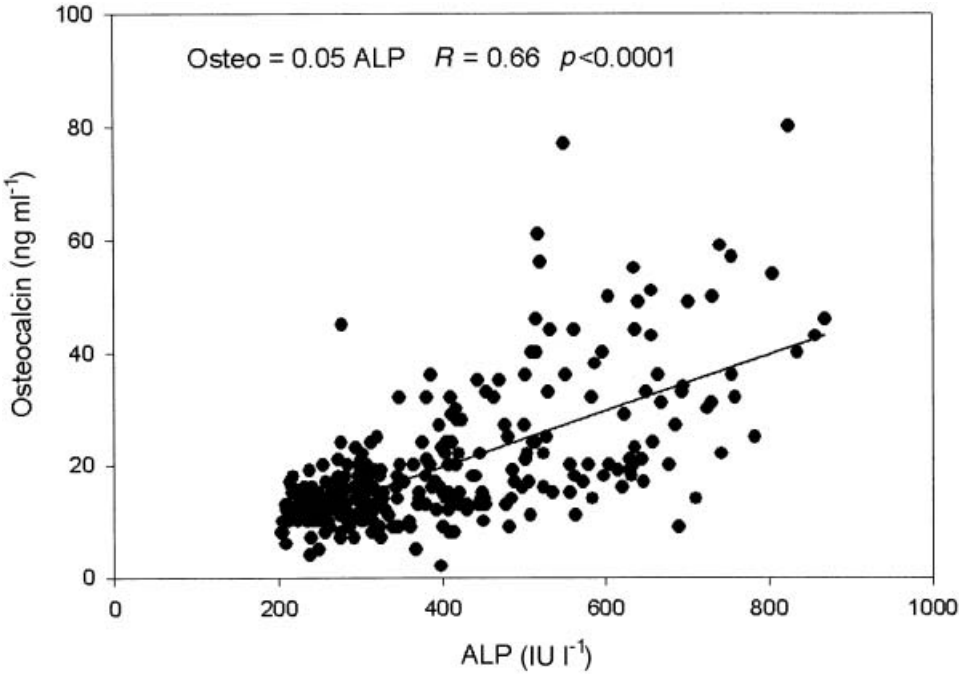

Figure 7. Correlation between total ALP activity and OC in SHAM and OVX old female cynomolgus monkeys.

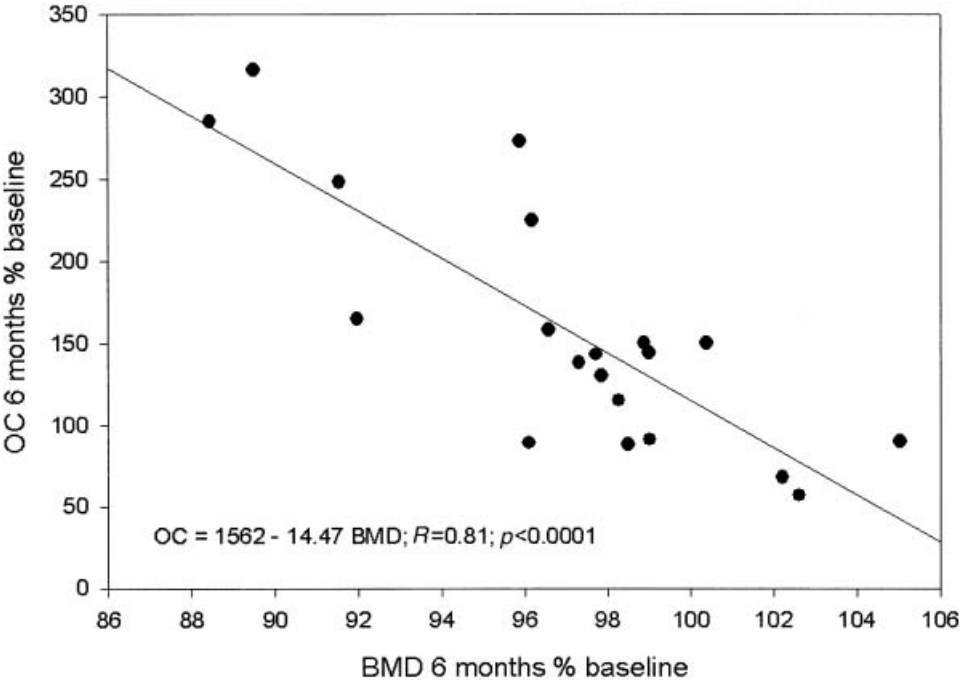

Figure 8. Individual correlation between BMD and OC changes at 6 months when compared with pre-surgery values.

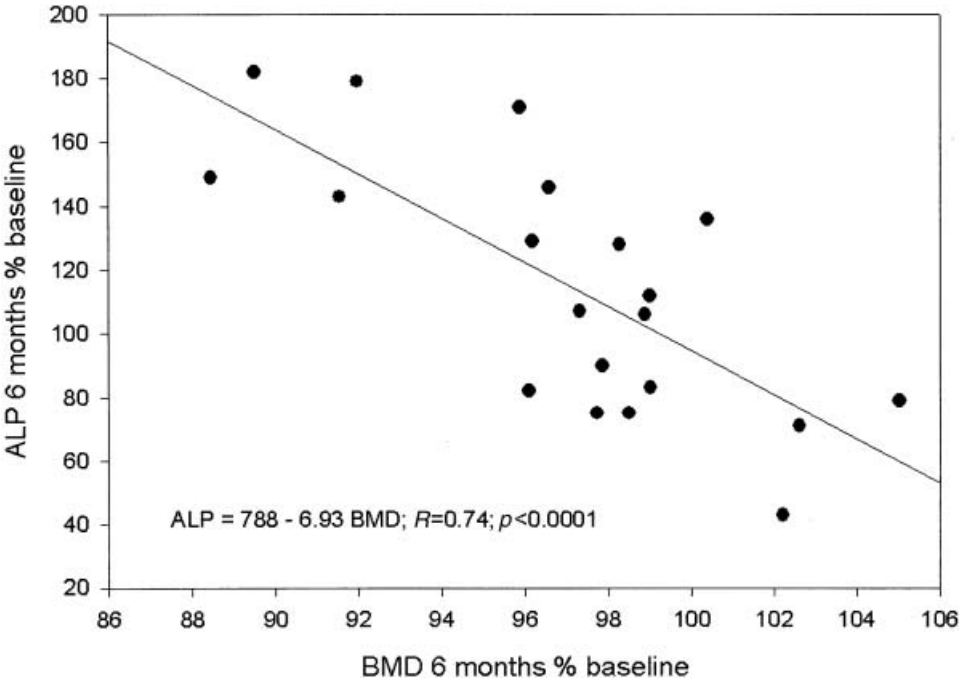

Figure 9. Individual correlation between BMD and total ALP changes at 6 months when compared with pre-surgery values.

1995). For the urinary markers (DPD and NTX-I) reported after normalization to creatinine, this variability is probably exacerbated by the day-to-day variation in creatinine in individual animals. However, even serum markers displayed great intra- and inter-individual variations that resulted in high standard deviations within the groups. As groups used for toxicology studies are usually limited to three to four monkeys per sex and per dose, and as some markers display variations between sexes, it may be useful to collect several samples from each animal over a few days and average their values to reduce the problem of the intra-individual variability.

Similarly to observations made in humans (Calvo *et al.* 1996) and in rhesus monkeys (Cahoon *et al.* 1996), baseline values were higher in young animals than in mature ones, reflecting the bone growth occurring in 2–3 year old cynomolgus monkeys. This correlation with age should be kept in mind when interpreting data obtained during toxicology studies, which are usually conducted in young animals. One notable exception is DPD, when the mean values did not differ between young and old female cynomolgus monkeys. However, as discussed below, this parameter was found to be insensitive to the induction of bone resorption in OVX cynomolgus monkeys and its validity in this species is therefore not established.

Although this was not examined in the present study, the influence of the menstrual cycle on bone turnover parameters should also be considered in intact female cynomolgus monkeys (Hotchkiss and Brommage 2000).

Osteopenia development after ovariectomy

The *in vivo* measurements of vertebral BMD in cynomolgus monkeys has been shown to be correlated, although slightly understating, to the vertebral mineral content measured by ash weight (Jayo *et al*. 1991). Animals with vertebral lesions were excluded from the study by radiographic screening before surgery, and the body weight evolution was similar between SHAM and OVX animals, excluding any influence of the body fat content as a possible cause of the BMD difference between the groups (Bolotin 1998). Although the animals were positioned for each DXA examination according to standardized procedures, a slight rotation of the vertebral column cannot be excluded and may have contributed to the intra-individual variation in this parameter and to the isolated increase observed in the OVX group at 13 and 16 months (Cheng *et al*. 2001). Excluding this isolated variation, the BMD followed a biphasic decrease after ovariectomy that reflects an abrupt change in the activation frequency of bone remodelling. Higher activation frequency at trabecular sites (Jerome *et al*. 1994, 1997a) and higher cortical porosity (Burr *et al*. 2001) have been reported in ovariectomized monkeys in comparison with age-matched sham-operated controls using histomorphometry analysis of bone samples 6 months to 2 years after surgery. The initial rapid drop in the BMD after ovariectomy is likely to represent a transient state, as defined by Parfitt (1980), where many new bone remodelling units are initiated, creating many new resorption lacunae. This transient phase lasts for approximately 6 months, corresponding to approximately the duration of two bone remodelling cycles, and is followed by a second phase where the BMD evolution apparently stabilizes as a result of the differential between the new resorption formation equilibrium. Similar patterns of BMD evolution have been observed when old, skeletally mature, animals are ovariectomized (Jerome *et al*. 1995), but our study demonstrated that a 10% decrease in BMD is achieved even when animals are fed a high-calcium diet. These findings are also similar to those observed in women after natural menopause or oophorectomy, when the BMD gradually decreases over a 5 year period (Krolner and Nielsen 1982, Stepan *et al*. 1987, Hreshchyshyn *et al*. 1988). This true osteopenia development contrasts with the relative osteopenia that is observed when younger animals (weighing 2.8–3.0 kg) are ovariectomized, and is characterized by a stabilization of the vertebral BMD relative to sham-operated animals, in which the vertebral BMD increases by approximately 10% during the months following surgery (Brommage *et al*. 1999b, Itoh 2002). Using such bone-growing animals may lead to misinterpretation of potential treatment actions.

Biochemical parameters during osteopenia development after ovariectomy

Only four parameters were repeatedly measured before and up to 20 months after surgery in the old female monkeys, namely total ALP, OC, PICP and DPD. Two other parameters (bone ALP and NTX-I) were only measured 16 and 20 months after surgery. The others were not available at the time of this study and remain to be validated in this model.

DPD is a small molecule that is released through the degradation of collagen during bone resorption. The lack of difference between OVX and SHAM animals after surgery is surprising as the BMD decrease clearly indicates an increase in bone resorption. Increases in this parameter have been reported after ovariectomy

in rhesus monkeys (Balena *et al*. 1993) and cynomolgus monkeys (Register and Jerome 1996, Itoh 2002) when quantified using high performance liquid chromatography (HPLC) (Black *et al*. 1988). Increases in DPD in rat urine samples were also observed in ovariectomized rats in our laboratory, using the same EIA as the one used in our study (Fisch *et al*. 1998b). However, no statistical difference between ovariectomized and sham-operated cynomolgus monkeys were observed for DPD when assessed by the EIA, although weak ($p < 0.1$) statistical significance was reached by measuring this parameter by HPLC in the same samples (Register and Jerome 1996). As DPD is a small molecule with the same chemical structure across species, a lack of cross-reactivity with the EIA in cynomolgus monkeys is unlikely, but this assay should be considered as insensitive for the detection of bone resorption in this species. Similar discrepancies between the HPLC and EIA measures of DPD have been reported in postmenopausal women, underlining the relative insensitivity of the EIA assay (Calvo *et al* 1996).

In the present study, urinary NTX-I was statistically higher in OVX monkeys 16 and 20 months after ovariectomy. As bone turnover is still higher at this time in ovariectomized monkeys compared with age-matched sham-operated animals (Jerome *et al*. 1994, 1997a), it is likely that this reflects increased bone resorption in these animals. Although we did not measure CTX-I in the urine of our OVX monkeys, no statistical differences were observed between ovariectomized and sham-operated cynomolgus monkeys 6 and 12 months after surgery by Hotchkiss *et al*. (2001) in an experiment including 27 animals in each group, despite the report by Register and Jerome (1996) of an earlier increase in this parameter after ovariectomy.

Circulating ALP activity is derived from several cell types and tissues but is mainly from liver hepatocytes and bone osteoblasts (Van Hoof and De Broe 1994). In normal animals, whether ovariectomized or not, total ALP activity measured by enzymatic assay is highly correlated to bone ALP activity measured by EIA. Both markers strictly follow the changes in osteoblast activity and are therefore equivalent markers of bone formation. However, discrepancies between total ALP and bone ALP measurements have been reported by Brommage *et al*. (1999a) in cynomolgus monkeys suffering liver diseases, since one ALP isoenzyme is synthesized by the liver. The measurement of bone ALP activity may be more reliable if the treatment administered to animals induces changes in the metabolism of the liver, intestine or kidneys.

Both ALP and OC are proteins secreted by osteoblasts (Delmas 1993). However, OC is subsequently incorporated into the bone matrix (Price *et al*. 1976). In cynomolgus monkeys, OC has been demonstrated by immunolocalization to be restricted to bone cells and mineralized bone matrix and to be lacking in non-calcified osteoid tissue (Carlson *et al*. 1993). Therefore, circulating OC may come directly from osteoblast secretion or may be released during the resorption of the matrix, and this parameter should be considered as a marker of global bone turnover rather than a specific marker of bone formation (Khosla and Kleerekoper 1999). This may account for the relatively poor correlation of OC and ALP. Statistically significant higher levels of OC (Weaver *et al*. 1994, Jerome and Lees 1996, Jerome *et al*. 1997b, Hotchkiss *et al*. 2001, Itoh *et al*. 2002), total ALP (Weaver *et al*. 1994, Jerome and Lees 1996, Jerome *et al*. 1997b) and bone ALP (Hotchkiss *et al*. 2001) have been previously reported in ovariectomized cynomolgus monkeys compared with sham-operated animals 6 months to 2 years after

surgery. Increased levels of OC have also been reported in female cynomolgus monkeys 6 and 9 months after the start of chemical castration by a gonadotrophin-releasing hormone (GnRH) agonist, with a return to normal pretreatment values 3 months after treatment cessation (Mann *et al*. 1992). Although this was not observed in our study, it should be kept in mind that OC is excreted by the kidneys and that elevated serum levels can occur secondary to spontaneous or drug-induced renal failure.

The relative insensitivity of PICP to increases in bone turnover in ovariectomized animals may be related to the lack of specificity of the assay, as it also measures PICP and procollagen III carboxy-terminal propeptide (PIIICP) produced from soft tissue synthesis. In patients with osteoporosis, this parameter was only poorly correlated with histomorphometric indices of bone formation and did not correlate with spinal BMD (Hassager *et al*. 1991).

In our study, both total ALP and OC changes (expressed as a percentage of baseline pre-surgery values) were highly correlated with BMD changes in OVX or SHAM old monkeys, demonstrating that both parameters are reliable parameters of bone turnover in this species.

Conclusion

The cynomolgus monkey is a unique animal model that may be used to assess the potential toxicity and efficacy of drugs intended to prevent or treat human osteoporosis in the same bone-remodelling species. Biochemical markers that can be measured rapidly, reproducibly and relatively inexpensively in the cynomolgus monkey are now available. They potentially provide an insight into osteoclastic bone resorption and osteoblastic bone formation, the two components of bone turnover, and can be similarly measured in humans. This is a significant advance for the preclinical assessment of the efficacy and safety of potential therapeutic agents. We have demonstrated that DPD measured by EIA was not sensitive enough to monitor the bone resorption induced by ovariectomy in this animal species. The large inter-individual variations observed for PICP makes this parameter relatively insensitive to changes in bone turnover when small number of animals are involved in the study. The bone turnover in ovariectomized old cynomolgus monkeys developing true osteopenia may be adequately monitored by the quantification of total ALP or bone ALP, OC and NTX-I. Other new markers have recently been made available but their usefulness remains to be validated.

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