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Prostatic acid phosphatase in the serially transplantable human prostatic tumor lines PC-82 and PC-EW*

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Summary. The correlation between tumor volume of untreated tumor-bearing nude mice and serum concentration of prostatic acid phosphatase (PAP/RIA) was studied in the hormone-dependent serially transplantable human prostatic tumor models PC-82 and PC-EW. The normal serum level of PAP in control male nude mice without tumor was found to be 0.9 ± 0.3 ng/ml. Elevated PAP serum concentrations were never found in animals without tumor (a highly specific diagnostic technique). A close correlation was observed between the concentration of PAP in the serum (range 0.3 to 154 ng/ml) and the tumor volume (range 10.0 to 6, 530 mm^3) of 104 untreated mice bearing a PC-82 or PC-EW human prostatic tumor. This correlation was comparable in both tumor lines ($p < 0.001$). The positive effect of endocrine manipulation which resulted in tumor diameter decrease or growth arrest with regressive histological patterns, showed the normal PAP serum level, too. After successful treatment PAP was found to be normal, independent from the residual tumor mass. By contrast, in the event of only retarded tumor growth, the PAP level still correlated with the tumor burden.

Key words: Nude mice – Human prostatic tumor models – PC-82 – PC-EW – Prostatic acid phosphatase

The nude mouse has been demonstrated to be a useful species for studies of human prostate cancer. The serially transplantable human prostatic carcinoma lines PC-82 and PC-EW were developed through heterotransplantation of tissue fragments from a cribriform prostatic carcinoma (PC-82) removed by total perineal prostatectomy ($pT_3pN_0M_0G_2$) and by transplanting tumor tissue (PC-EW) from a lymph node metastasis ($cT_3pN_2M_0G_3$) [10–12]. Both nude mice tumor lines are androgen dependent and similar to the original tumors in their histological pattern. They secrete PAP that is detectable in the serum of tumor-bearing animals [7, 14].

The concentration of PAP is often elevated in the serum of patients with prostate cancer. Determination of human PAP activity in the serum has been widely used to detect prostatic carcinoma and to monitor therapy [8, 13]. Therapeutic measures which result in a decrease of circulating androgen and in a regression of disease often also give rise to a decrease in the concentration of PAP in the circulation.

Our androgen dependent tumor models are useful for the study of correlation between the PAP level in the serum and the tumor burden of the mice and for studying the effect of short-term endocrine manipulation in vivo on the PAP concentration in the peripheral blood of tumor-bearing animals. The comparative study of two serially transplantable tumor lines offers a unique model for the experimental research of tumor markers.

Material and methods

Tumor material

Male nu/nu nude mice of Balb/c origin received a transplant of 2 to 4 mm^3 particles from PC-82 or PC-EW tumor at an age of 8 to 10 weeks. The fragments were grafted surgically into the dorsal subcutaneous space of the animals under ether anesthesia. This subcutaneous insertion technique for tumor grafts gave a take rate of 90% (Fig. 1).

Measurements

The microscopic tumor size was determined by means of a slide caliper with a dial gauge. Two diameters of the developing tumors, i.e., the largest diameter and the one perpendicular to it were monitored daily. Tumor volume was calculated according to the formula established by van Steenbrugge et al. [14]. For the experiments, tumors from the 30th-35th (PC-82) and 19th-24th (PC-EW) mouse transplant generations were used.

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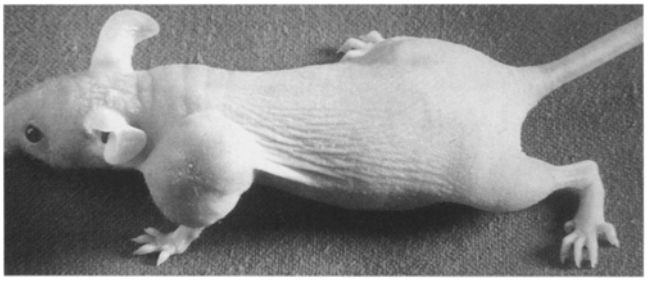

Fig. 1. PC-EW tumor-bearing nude mouse with large tumor in the left dorsal subcutaneous space

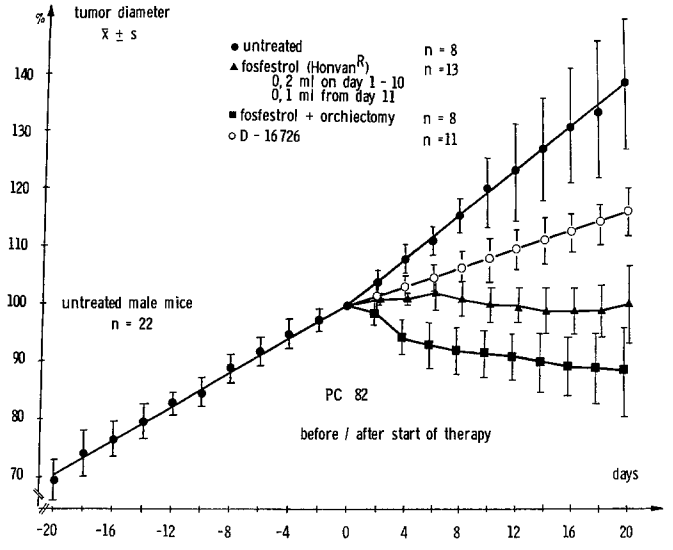

Fig. 2. Diameters of untreated and treated PC-82 tumor during the exponential growth phase. Tumor diameter on the first day of therapy calculated to be 100% (standard deviations are indicated)

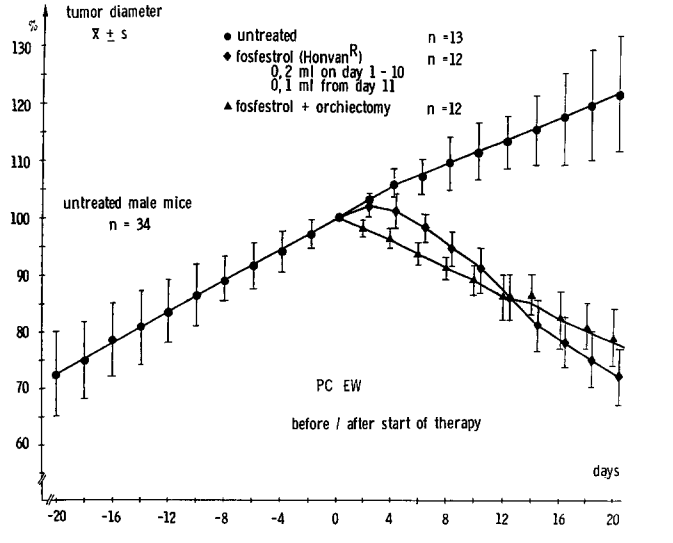

Fig. 3. Diameters of untreated and treated PC-EW tumors during the exponential growth phase. Tumor diameter on the first day of therapy calculated to be 100% (standard deviations are indicated)

Treatment of the animals

Treatment studies were started during the exponential growth phase of the tumors. The absolute measured values were converted into percentages, and later, when therapy was applied, the tumor diameter was taken to be equivalent to 100 percent. This allowed a direct comparison between the various tumors and the efficacy of the different therapy modalities. Thus it became possible for the growth curves of all tumors treated to be shown in a single figure for direct comparison (Figs. 2 and 3). Orchiectomy was carried out via the scrotal route under ether anesthesia. Therapy consisted in giving different doses of fosfestrol (1.2 mg on day 1 to 10 and 0.6 mg from day 11) and of the substance D-16726 (a phenyl-indol-derivate) 8.0 mg/kg three times per week were administered intraperitoneally for 20 days, on average.

Serum specimens

Animals were divided into 5 groups. Group 1 ($n = 16$): intact male nude mice (without tumor). Group 2 ($n = 36$): tumor-bearing male nude mice with untreated PC-82 tumor. Group 3 ($n = 68$): tumor-bearing male nude mice with untreated PC-EW tumor. Group 4 ($n = 14$): tumor-bearing male nude mice (PC-82 or PC-EW), which were castrated or treated with fosfestrol. Group 5 ($n = 11$): tumor-bearing male nude mice treated with D-16726. Tumor-bearing animals without therapy (Groups 2 and 3) were killed at various points in time after transplantation. The serum values of PAP were determined in these animals with different end volumes of the tumor nodules. The serum parameters of the castrated and hormone-treated mice were examined after a treatment period of 20 days on average. Blood was withdrawn after cervical section by opening the carotid arteries under ether anesthesia. In order to prevent hemolysis, the blood was briefly cooled down and the serum was obtained by centrifugation for 2 min at 6000 rpm. The serum was deep-frozen or analyzed on the same day.

Radioimmunoassay procedure

For the quantitative determination of PAP levels in serum the GammaDab (125-I) PAP Radioimmunoassay Kit (Travenol Genentech Diagnostics) was used. This procedure is a competitive binding assay which utilizes a precipitating antiserum reagent to separate antibody-bound tracer from unbound tracer. A standard curve with four serum standards ranging from 1 to 30 ng/ml was used. The PAP concentrations of the samples were interpolated from the standard curve. In case of high values, the sera were diluted up to tenfold.

Other procedures

The histological examination was based on routine techniques. Statistical analysis of the data was performed by linear regression analysis.

Results

PAP in serum of control animals (Group 1)

As controls the serum values of 16 male nude mice without tumor were determined. A readily measurable PAP value of 0.9 ± 0.3 (range 0.2 to 1.6) ng/ml was found. For the test animals a normal upper limit of 1.8 ng/ml ($x \pm 3 \times$ SD) was defined.

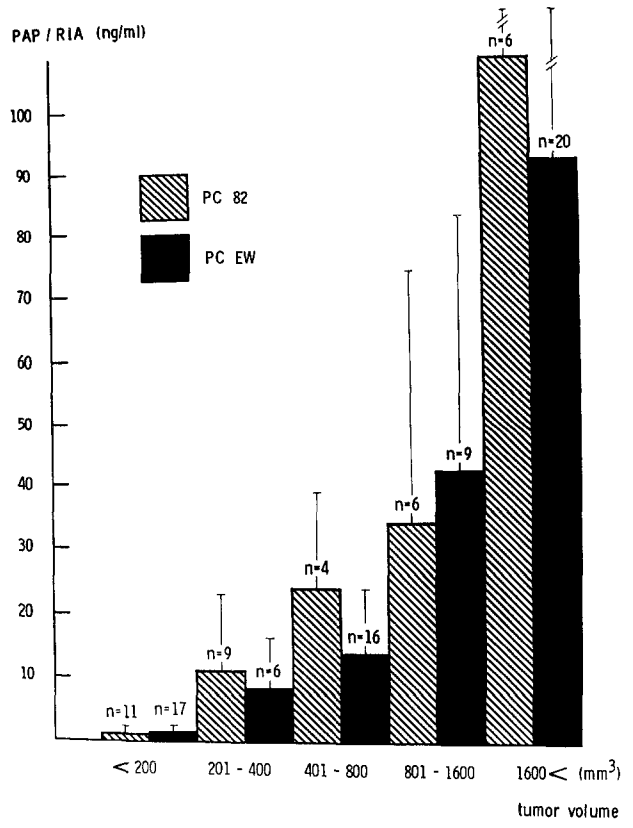

Fig. 4. Average serum values of PAP in relation to tumor volume groups in PC-82 and PC-EW tumor-bearing male nude mice without therapy

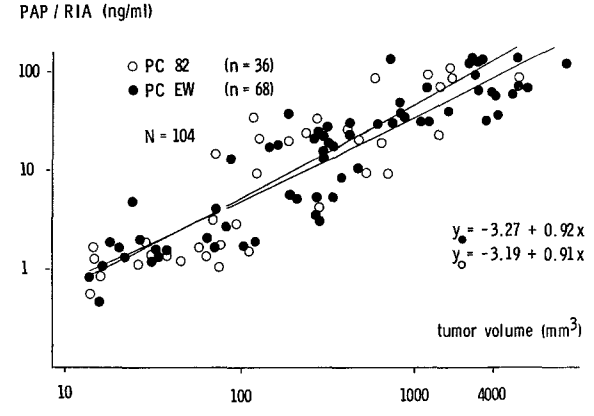

Fig. 5. Correlation of serum PAP with actual tumor volume in untreated nude mice bearing PC-82 and PC-EW tumor

PAP in serum of tumor-bearing male nude mice without therapy (Groups 2 and 3)

The data of the tumor lines of different human origin were evaluated separately. With a total of 104 animals investigated, it was possible to determine the serum level of PAP within a broad tumor volume spectrum from 10.0 to 6,350 mm^3. In 28 cases the tumor size was smaller than 200 mm^3, in 15 animals it varied between 200 and 400 mm^3, in 20 cases between 400 and 800 mm^3, in 15 animals it ranged from 800 to 1,600 mm^3 and in 26 cases it was over 1,600 mm^3 (Fig. 4). In this volume spectrum the PAP value was true-positive, i.e., over the 1.8 ng/ml limit in 73 animals (70%). The 31 false-negative findings (= 30% of the total figure) were established among the first 38 animals (82%) with a tumor volume up to 315 mm^3. In 48/104 mice (46%) the value was more than 10 times higher than the normal upper limit (1.8 ng/ml) in 28/104 cases (27%) the value exceeded the limit by more than 20 times and only in 14/104 animals (13%) was the PAP level over the 50 times limit. The highest PAP value was 154 ng/ml which means an 86-fold increase.

The data for the tumor lines of different human origin are shown, separately evaluated, in Fig. 4 and 5. A close correlation was observed between the concentration of PAP in the serum (range 0.3 to 154 ng/ml) and the tumor volume of the untreated mice bearing 36 PC-82 and 68 PC-EW human prostatic tumors. Correlation of serum PAP and tumor volume was comparable in both tumor lines. Figure 5 shows that the linear regression lines of the PAP values as a function of the tumor volume are identical.

Influence of different treatment modalities on the PAP concentration in the serum of tumor-bearing animals (Groups 4 and 5)

Twenty days after the beginning of therapy, the PAP serum values were determined in 25 tumor-bearing mice with different tumor growth as shown by the growth curves in Figs. 2 and 3. (The blood samples could not be examined in all animals under treatment, which were measured for the presentation of the growth curves. Some animals had not enough serum for a quantitative analysis, others died during the night or were lost for other reasons). Fourteen animals were studied after fosfestrol-therapy alone or fosfestrol combined with castration and another 11 mice were treated with the D-16726 substance. As among the untreated animals there was no difference in the PAP level/tumor volume relationship, the various tumor lines were no longer dealt separately with regard to the behavior of PAP serum values during the therapy studies. In comparison with the untreated animals, an effect was ascertained in all tumors measured. However, the growth curves reveal a clear difference among the various therapy groups. The animals treated with fosfestrol on a staged scheme based on an initial high dosage of 1.2 mg/day showed already after few days a primary delay in growth followed by an arrest of tumor growth or regression. This phenomenon is more apparent with the PC-EW tumor, than with the PC-82 line. The substance D-16726, which is not used in clinical urology, obtained only delayed growth of the PC-82 tumor in response to a dose of 8.0 mg (Fig. 2). This effect was followed up over a period of 20 days and was corroborated by a prolonged observation period of up to 50 days in $n = 9$ tumors. In the animals treated, histological signs of regression were almost invariably identifiable, as soon as their growth curve followed a course below that of the untreated group, i.e., already in the case of delayed growth caused by D-16726. Figure 6 shows histology of the PC-82 (a) and

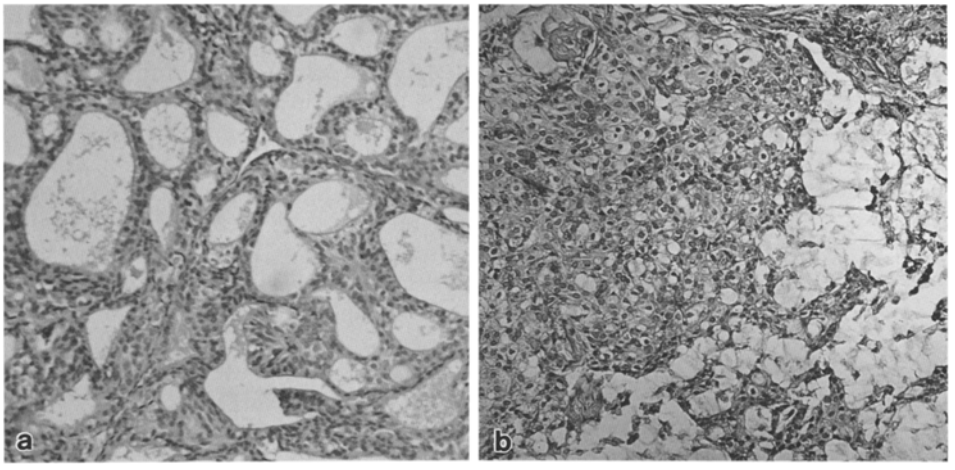

Fig. 6. PC-82 (a) and PC-EW (b) tumors 20 days after fosfestrol therapy. Regressive changes include pycnotic nuclei, less distinct nucleoli, vacuolized cytoplasma, widened acinar lumina (×255)

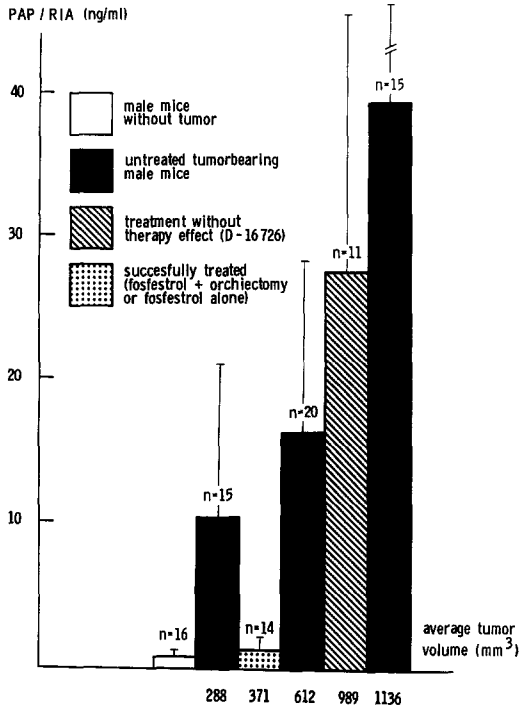

Fig. 7. Mean serum concentrations of PAP in tumor-free and tumor-bearing male nude mice with (hatched and dotted columns) and without (black columns) therapy

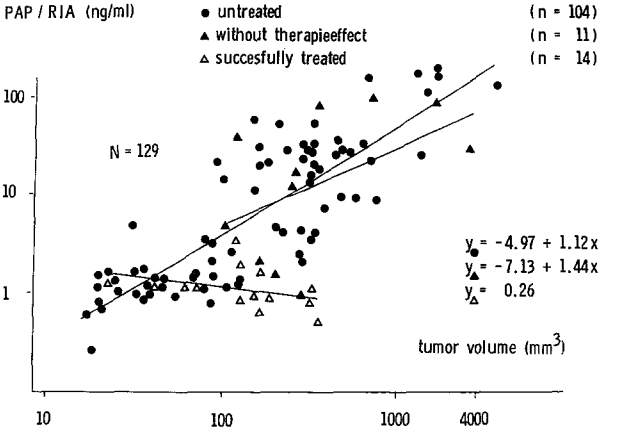

Fig. 8. Correlation of serum PAP with the actual tumor volume in untreated, successfully treated and unsucessfully treated animals

PC-EW (b) lines after fosfestrol therapy (dilated glandular lumen, irregular flattening of the epithelium, vacuolization of the cytoplasm, individual cell hornification and small necrotic lesions).

The treatment with fosfestrol alone or combined with castration brought the PAP values back to the normal range after 20 days, independent of the residual tumor volume, which testifies to the biochemical inactivity of the tumor. The average PAP value was 1.4 ± 0.7 ng/ml with an average residual tumor volume of 371 ± 228 mm^3. By contrast, the related results achieved with the D-16726 substance in the PC-82 tumorline, were different. Here a delayed growth and moderate histological regression were observed and the PAP values (16.1 ± 12.0 ng/ml) remained elevated in correspondence to the prevailing effective tumor volumes (612 ± 85 ng/ml) (Figs. 7 and 8). The columns in Fig. 7 show the mean serum concentrations of PAP in tumor-free and tumor-bearing male nude mice. The black columns distinctly demonstrate that, if no therapy is instituted, also the serum value of the tumor marker increases with the growing tumor volume. If therapy proves unsuccessful, the PAP value which remained elevated, corresponds to the increasing tumor volume (hatched column). In contrast to that, the tumor marker level is in the normal range (dotted column) independent of the residual tumor volume, following successful fosfestrol application and castration.

Discussion

With the human carcinomas PC-82 and PC-EW two representative tumorlines heterotransplanted on the nude mouse are available, which both, as to hormonal dependence and tumor marker production, come very close to the

in-situ behavior in humans and thus can be looked upon as suitable animal models for the investigation of the prostate carcinoma [7, 10–12]. These tumors have take-on rates up to 90% and conform to the original material during the animal passages. Their hormonal dependence manifests itself by a missed take-on female and castrated animals and by a tumor regression in response to an efficient androgenoprivic therapy. The advantages of the nude mouse tumor models are: (1) Better representation of the human tumor-specific properties and observation of the histological degree of regression, (2) possibility of studying in vivo the therapeutic effect of castration, also in conjunction with the administration of estrogens, antiandrogens or cytostatic drugs, (3) agreement with the clinical course of therapy in the "donor" patients with regard to both the response of the local prostatic carcinoma (PC-82) and the bone metastases (PC-EW) and (4) these models are suitable for tumor marker studies and for monitoring therapy success.

The enzymatic activity or the antigenic concentration of serum PAP may be elevated in patients with adenocarcinoma of the prostate. It was the first prostatic cancer marker to be reported [9]. Cancerous prostate tissue produces more PAP than healthy tissue. This enzyme is concentrated both in the epithelium of the primary lesion and in the metastases of the prostate carcinoma. From the fundamental research done by Foti and associates [8] it emerged that PAP can also act as antigen. The discovery of the organ-specific antigenicity of PAP led to the development of various measuring methods which have unequivocally improved the specificity of PAP determination in the diagnosis of the prostate cancer [2–4, 15]. However, the procedure has not yet satisfied the requirement of providing a tool for early tumor detection [16]. PAP is reasonably sensitive for detection of stage D prostatic cancer, but it is less sensitive in the A, B and C disease stage [1]. Acid phosphatase activity in the prostate gland is localized primarily in the glandular and ductal epithelia.

In our study we report on experimental investigations of the behavior of serum PAP values in the human nude mouse prostate carcinoma lines PC-82 and PC-EW during uninhibited tumor growth as well as under androgenoprivic therapy. The present data from the PC-82 and PC-EW tumor models are in agreement with the concept that the PAP concentration in serum is related to the tumor mass. A close correlation was observed between the total tumor volume and the PAP level in the serum of both tumor lines. Our results are similar to the data of van Steenbrugge and associates [14]. They have established a significant correlation between the serum concentration of PAP and the total tumor load in PC-82 tumor-bearing mice. Furthermore, they have shown that castration of tumor mice did not result in a decrease of PAP in the serum after 5 days, while over a longer period castration leads to a significant reduction of both the mean tumor volume and the PAP concentration in the blood circulation. Our study revealed also, that serum values of PAP are dependent on the tumor volume. Admittedly, with a certain tumor size (315 mm^3), the proportion of false-negative values (82%) was rather high.

In animals treated with different modalities in cases of histologically and growth-kinetically verified tumor regression, the PAP serum level points to a decreasing or arrested tumor activity. Arrest of growth and/or decrease in volume were associated with falling PAP values (often within the normal range), and with regressive histology.

Comparison with the untreated control animals revealed that an effect was produced in all tumors measured. However, the growth curves showed, apart from slight modifications in both tumor lines, a clear difference among the individual therapy groups.

Basically, the following phenomena can be distinguished: (1) uninhibited growth, (2) delayed growth, (3) arrest of growth and (4) tumor regression. It is apparent from the growth curves (Figs. 2, 3) that the treatment with fosfestrol alone or combined with castration had a distinctly stronger impact on the PC-EW line (pronounced regression of tumor size) than on the PC-82 line (arrest of growth or slight regression of tumor volume). However, the PAP values of both lines were back in the normal range after already 20 days, independent of the tumor volume, and this pointed to the biochemical inactivity of the tumor (Fig. 8). The delayed growth and the moderate histologic regression achieved with the D-16726 substance were accompanied by elevated PAP values according to the prevailing tumor volume. No significant difference was found in the straight line of regression for the tumor volume-dependent PAP values between the animals treated with this preparation and the untreated tumor-bearing animals (Fig. 7, 8). This means that D-16726 based therapy must be classified as unsuccessful, despite the delayed growth obtained. This result would also require that in future studies of the response to therapy a tumor-marker determination must be included. If no signs of biochemical inactivity are identified, therapy should not be deemed successful, even if growth was delayed. The comparison between the growth curves mentioned and the PAP level allows us to conclude that arrest of growth or tumor regression go hand in hand with a significant inhibition of the tumor activity.

In contrast, merely delayed tumor growth with elevated PAP values would again indicate the vitality of the tumor. These results coincide with clinical observations based on the assumption that with increasing inactivity and regression of the tumor the release of phosphatase decreases [1, 6, 13]. Consequently, for the assessment of the therapeutic success in nude mouse studies, a determination of the PAP (and in future also of the prostate specific antigen (PSA) because of its higher sensitivity!) is necessary, in addition to the metric and histologic evaluation.

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