Dexamethasone Reduces the Interstitial Fluid Pressure in a Human Colon Adenocarcinoma Xenograft

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Abstract

The effect of dexamethasone on interstitial hypertension was evaluated in a human colonic adenocarcinoma. Two weeks after transplantation of the tumor line LS174T into SCID mice, recipients with tumors >8.5 mm in diameter received one daily injection i.p. on days 1–4, at five different doses in the range of 0.3–30 mg/kg. Controls received saline. The interstitial fluid pressure (IFP) was determined in all tumors pretherapeutically on days 1, 4, and 7. A total of 68 tumors were examined, and in an additional group of 22 mice, the effect of 4-day dexamethasone therapy on blood pressure was evaluated. In the 3-, 10-, and 30-mg/kg dose groups a significant reduction in IFP was found, comparing treated versus controls and individual measurements from day 1 versus day 4. No effects were observed on day 7. A marginal effect was observed after 1.0 mg/kg, whereas 0.3 mg/kg did not affect the IFP. The systemic blood pressure was slightly increased by the dexamethasone therapy, and no treatment-related changes in tumor sizes were observed. Our findings indicate that the reversible decrease in tumor IFP by dexamethasone is an effect of a reduced microvascular permeability and vascular hydraulic conductivity in the tumors.

Introduction

Whereas normal tissues tend to have an IFP2 close to zero mm Hg, an elevated IFP is found in all examined experimental and human tumors (1–7). The etiology of the ubiquitously elevated tumor IFP is not fully elucidated but the following factors are thought to be involved: (a) the absence of functional lymphatics in tumors; (b) the abnormally high permeability of tumor vessels; and (c) an increased propensity for microthrombus formation, as well as extravascular occlusion, in tumor vasculature (1, 8). Consequently, the hydrostatic pressures in the vascular and interstitial spaces are approximately similar, as recently demonstrated in a tissue-isolated rat tumor (8). In theoretical and experimental models (1, 9), the resulting lack of a hydrostatic pressure gradient across the vascular wall constitutes a major obstacle for delivery of macromolecules into solid tumors. Therefore, there is an increasing interest in methods to actively modify the interstitial hypertension. Nicotinamide (500 mg/kg i.p.) has been shown to acutely lower the IFP in a murine tumor (10). Recently in another murine tumor, heparin (100 IU s.c. daily for 7 days) as well as warfarin (1 mg/kg p.o. daily for 7 days) was reported to produce significantly lower IFP in treated compared to untreated controls (11). A decrease in tumor IFP in responding tumors has been shown in cervical carcinoma during radiotherapy (4) and in lymphoma following combination chemotherapy (6).

In brain, interstitial hypertension, i.e., vasogenic edema, associated with tumors or trauma is effectively reduced and symptomatically palliated by dexamethasone or related glucocorticoid steroids in clinical and experimental settings (12–16). The present study was undertaken to examine whether dexamethasone exerts a similar effect on the interstitial hypertension in solid tumors.

Materials and Methods

The human colon adenocarcinoma LS174T was chosen because we have characterized previously several physiological parameters in this tumor and have obtained uniformly high IFP values in repeated series (17). LS174T was transplanted s.c. under aseptic conditions in the right hind leg of male SCID mice, 8 weeks old, weighing 22–25 g. Experiments were started when the tumors reached a diameter of ~8.5 mm. This was achieved after 12–15 days of growth. Dexamethasone was administered in 0.9% saline, 0.1 ml/10 g body weight, i.p. daily on days 1–4, at 5 different doses, ranging from 0.3 to 30 mg/kg (Table 1). Controls received corresponding volumes of saline i.p. IFP was measured three times in each tumor: (a) prior to therapy on day 1; (b) 36 h following the last injection on day 4; and (c) 3 days posttherapy on day 7. Prior to measurements, the mice were anesthetized with a s.c. injection of ketamine (10 mg/kg) and xylazine (1 mg/kg) in a saline solution. Tumor IFP measurements were performed by the wick-in-needle method, and MABP was measured by inserting a PE-10 cannula in the left carotid artery in separate groups of nontumor-bearing animals, treated according to the same protocol and in untreated controls. The detailed experimental procedures have been described previously elsewhere (8, 17). The weight of the mice and the tumor size were measured on days 1, 4, and 7. Institutional guidelines for conduct and animal welfare were followed.

Results and Discussion

The tumor IFP data at each dose level are summarized in Table 1. A significant decrease in tumor IFP was observed following 4-day therapy with 3-, 10-, and 30-mg/kg daily dexamethasone injections i.p. The difference in IFP was statistically significant both when comparing treated versus controls on day 4 and when comparing measurements in the same tumors from days 1 and 4. The decrease was temporary, since on day 8, after 3 days of recovery, there was no significant difference between the IFP of treated animals and controls and no significant difference from day 1 values. A borderline effect was observed at the lower dose of 1.0 mg/kg, whereas no effect was observed following 0.3 mg/kg. The absolute decrease in IFP after a 4-day course of dexamethasone therapy was almost identical in the effective dose levels as shown in Table 2; thus no indication of a continuous dose-response relationship in the 3–30-mg/kg range was found. There was no significant difference in the tumor growth in treated animals and controls, nor was any significant change in body weight observed in any group. In each treatment group some tumors were eaten by the host animals or their peers and, therefore, excluded from the analysis. This phenomenon, which did not occur in controls, might cautiously be interpreted as steroid toxicity. Natural and synthetic glucocorticoids can produce hypertension in humans and, correspondingly, a slightly increased MABP was seen in dexamethasone-treated animals (10 mg/kg daily for 4 days) when compared to controls (Table 3). With a stable or increased systemic blood pressure, a reduction in steady state MVP is not likely to occur, unless a significant increase in the precapillary resistance is produced by dexa.
Lp is the hydraulic conductivity of the vascular wall (1). If we assume vascular and interstitial oncotic pressures, Jv is the filtration rate, and tumor blood flow in LS174T (17) has been shown to produce a concomitant increase in the tumor IFF and methasone, as suggested by Tajima et al. (15). However, vasoconstriction and an increase in MABP, induced by angiotensin II, have been shown to produce a concomitant increase in the tumor IFF and tumor blood flow in LS174T (17).

According to Starling's law, the IFF is given by

$$ IFF = \frac{MVP}{\pi_s - \pi_i} - \left( \frac{J_v}{L_p} \right) $$

where $\sigma$ is the osmotic reflection coefficient, $\pi_s$ and $\pi_i$ are the vascular and interstitial oncotic pressures, $J_v$ is the filtration rate, and $L_p$ is the hydraulic conductivity of the vascular wall (1). If we assume that the MVP was unchanged, two factors can decrease the IFF in tissues: (a) an increased oncotic pressure difference ($\pi_s - \pi_i$) between the vascular and interstitial space; and (b) an altered ratio, $J_v/L_p$, of fluid flux relative to the hydraulic conductivity of the vessel wall. In untreated tumors the value of ($\pi_s - \pi_i$) seems to be low, probably due to the leakiness of tumor vessels. This was indirectly evidenced by the demonstration of Boucher and Jain (8) that in tissue-isolated rat adenocarcinoma the MVP and IFF are almost equal and by Sylven and Bois (18), who found protein concentrations in the interstitial fluid ranging between 67 and 97% of the plasma concentration in various murine tumors. Gullino et al. (19), however, found larger differences between interstitial and plasma protein levels. More directly, a relatively high vascular permeability has been demonstrated in several tumor models, including LS174T (20, 21). Based on these considerations, the most plausible mechanism behind the observed reduction of IFF following dexamethasone therapy is a drug-induced decrease in the microvascular permeability and vascular hydraulic conductivity, $L_p$, leading to an increased ($\pi_s - \pi_i$) and $J_v/L_p$. This mechanism finds support in previous observations and clinical experience with dexamethasone and related steroids. Reichman et al. (22) saw a decrease in vascular permeability in rat glioma not only by dexamethasone and methylprednisolone but also by the nonsteroid agents ibuprofen and indomethacin. In the recent study by Curti et al. (11), however, there was no effect of indomethacin (or aspirin) on the tumor IFF, which might suggest that a change in the vascular permeability does not invariably lower the IFF. Hyperglycemia induced by dexamethasone may have contributed to an osmotic gradient, but this would occur only secondary to a pronounced decrease in the vascular permeability even for glucose; otherwise the glucose would freely diffuse into the interstitial tissue.

Since elevated IFF has been suggested as a major obstacle to macromolecular delivery, it would appear that lowering IFF relative to MVP by dexamethasone should increase the convective transfer of molecules from tumor vessels. However, the concomitant decrease in vascular permeability may counteract this effect by a reduced diffusive transfer. This hypothesis is in concert with observations that dexamethasone treatment can reduce the delivery (i.e., extravasation) of large molecules, e.g., serum albumin (22), and also smaller compounds such as methotrexate (23) in tumors.

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References


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