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Interstitial fluid pressure as an obstacle in treatment of solid tumors

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Over the past decades a development of different anticancer drugs has increased and brought many progressive agents that showed high level of efficiency in in vitro conditions. Unfortunately these drugs failed in solid tumor treatment in in vivo conditions because of inadequate uptake and nonoptimal distribution in tumors. Although tumors have higher permeability and hydraulic conductivity of the vessels than normal tissue, the extravasation of the drug molecules from vessels into the tumor interstitium is reduced due to elevated interstitial fluid pressure (IFP). This property also impedes the transport of the molecules through the interstitial space. Furthermore, IFP is uniformly high in the center of the tumor and declines to the value of the normal tissue at the rim of the tumor. Though, IFP gradient causes fluid flow which "washes" drugs out of the tumor to its periphery where it is reabsorbed by the lymphatic system or normal vasculature. Measurements of tumor IFP demonstrated that its values can reach 2600 Pa up to 6600 Pa whereas in the normal tissue it is below the atmospheric pressure (from -133 Pa to -798 Pa in s.c. and approximately -346 Pa in muscle). The most frequently used methods for instant and direct IFP measurement are: wick-in-needle technique (WIN) and micropuncture technique (MP). Since the reduction of the elevated tumor IFP could facilitate drug uptake and anti-tumor treatment, many approaches have been tested. In present paper we represent the results of two physical (hyperthermia, radiation) and one chemical (vasoactive agents) approach that other authors used for IFP reduction.

Key words: neoplasms-therapy; extracellular space; manometry

Introduction

A high level of drug development techniques, especially genetic engineering, has produced many novel drugs for cancer detection and treatment.[1,2,3] The first step in the development of such a drug is *in vitro* testing and many agents showed a very high degree of anti-cancer effectiveness. This stimulated the use of low-molecular-weight conventional drugs, monoclonal antibodies, growth factors, biological response modifiers, immunotoxins, lymphokine activated killer cells, tumor-infiltrating lymphocytes, and others in *in vitro* conditions.[2] Although they succeeded in the treatment of leukemias and lymphomas they had a minimal effect on solid tumors (breast, colon, lung, ...).[2,3] The main reason for their limited effectiveness is inadequate and nonuniform distribution of drug molecules or cells in tumors.[2-5] Since cellular factors, such as heterogeneity of tumor-associated antigens and inherent or acquired tumor resistance can not explain this problem, physiological factors have to be concerned.[2] To complete its mission, molecule of the blood-borne anticancer drug must travel via blood stream to the tumor. Further it must extravasate across the microvessel wall into tumor interstitium, where it must disperse uniformly in the tumor in order to reach each tumor cell. All of these steps

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are not present in *in vitro* experiments so each of these physiological factors could be the reason for the ineffectiveness of anticancer drug.[2,3]

The role of interstitial fluid pressure in transport of molecules through microvessel wall and tumor interstitial space

As tumor cells proliferate into the host tissue, tumor angiogenesis leads to the formation of a new, tumor vasculature.[2,6] Although the tumor microcirculation originates from the normal host vasculature, its organization may be completely different and vary from day to day and from one location to another. Vessels in tumor are, compared to vessels in normal tissue, more dilated, sacular and tortuous. They can also contain tumor cells within the endothelial lining of the vessel wall. Furthermore, tumor microvessels have wider intercellular junctions and discontinuous or absent basement membrane. Another difference between the normal and the tumor vasculature is that the latter has a large number of fenestrae and blood channels which are not lined with endothelial cells.[2,7]

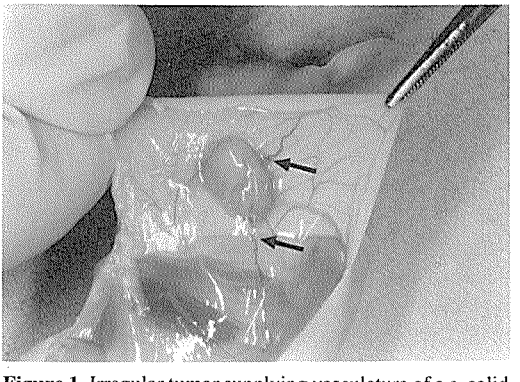

Figure 1. Irregular tumor supplying vasculature of s.c. solid LPB tumor in nude mice (arrows).

The extravasation of the blood-borne molecule that has reached the tumor vasculature is governed by diffusion and convection. The diffusion is a movement of the solute in the medium from an area with high concentration to an area with low concentration and is the primary way of transport for low-molecular-weight hydrophilic and lipophilic molecules. The diffusion is proportional to the concentration gradient and exchange vessel area. The proportionality constant that relates transluminal flux to the concentration gradient is the vascular permeability.[2,7] The convection on the other hand is a way of molecular transport by a stream of fluid. It is proportional to the difference between the vascular and the interstitial hydrostatic pressures minus the difference between the vascular and the interstitial osmotic pressures and also the exchange vessel area. Constants that relate fluid leakage to the pressure gradients are hydraulic conductivity for hydrostatic pressure difference and reflection coefficient for osmotic pressure difference. The equation that describes the solute flow across the vessel wall due to diffusion is:[7]

$$J_s = P \times A \times (c_v - c_i)$$

where: J_s is the flow of solute (moles/s or g/s); P is the vessel permeability (m/s); A is the surface area of the vessel (m^2); and c_v and c_i are the concentration within vessels and interstitial concentration of solute, respectively (moles/m^3 or g/m^3). The fluid flow across the vessel wall is given by:[7]

$$J_f = L_p \times A \times [\,(p_v - p_i) - \sigma \times (\pi_v - \pi_i)\,]$$

where: J_f is the volume flow of fluid (m^3/s); L_p is the hydraulic conductivity (filtration coefficient) of the vessel (m/Pa×s); A is the surface area (m^2); p_v and p_i are the vascular and interstitial fluid pessures (Pa); π_v and π_i are the colloid-osmotic pressures in vessel and interstitial fluid (Pa); and σ is the osmotic reflection coefficient. In the presence of convection and diffusion the total solute flow is given by the Staverman-Kadem-Katchalsky equation:[7]

$$J_s = P \times A \times (c_v - c_i) + J_f \times (1 - \sigma_F) \times \Delta c_{lm}$$

where: σ_F is the solvent-drag reflection coefficient; and Δc_{lm} is the log-mean concentration within the pore. For larger molecules the convection is the basic and a faster way of transport, although they also travel by diffusion.[2,3,7] Characteristics of tumor vessels described above suggest that they should have a relatively high vascular permeability and hydraulic conductivity. Various studies measuring tissue uptake confirmed that hypothesis.[2,7] Nevertheless, the extravasation of anticancer agents in solid tumors is poor. The main reasons are that, tumor does not create its own functioning lymphatic system, therefore, the excess fluid collects in the tumor interstitium and that tumor cells proliferate in the relatively limited, noncompliant space. These tumor properties cause increase of the interstitial fluid pressure (IFP). The elevated IFP hinders the convection across the vessel wall, because there is

no difference between the vascular and the interstitial pressure.[2-4,7] First it was assumed that the increased IFP causes the vessel occlusion in tumor, since IFP is higher than microvascular pressure (MVP). MVP relates to pressure in vessels with diameter 25 and 250 μm.[8,9] This hypothesis, however, failed to explain why a convection in the opposite way did not occur, i.e. fluid flow from interstitium into vessel. In addition, tumor vessels are very perfusive and though represent no resistance to the IFP.[7,8] Boucher and Jain demonstrated that MVP is increased and equal to IFP, furthermore, they assumed that MVP is the principal driving force for the elevated IFP.[8] The reasons for the observed increased MVP may be an increase in viscous and/or geometric resistance in the venous side of tumor circulation and that arterioles become less effective in controlling MVP.[8] Later they found out that the relationship between these two factors varies from one tumor to another.[9]

Another aspect in nonadequate anticancer agent uptake in tumor is the heterogeneity of the tumor vasculature. In general, solid tumors have three different regions: necrotic zone, semi-necrotic zone and well vascularized zone.[2] In necrotic and semi-necrotic zone there is no or very little blood supply and hence no extravasation of anticancer drug takes place. Tumor blood flow in these areas is also low compared to blood flow in normal tissue, whereas in well perfused zone (usually at the tumor periphery) tumor blood flow may be higher than that in normal tissue.[2] The increase of intercapillary distance and the decrease of vascular surface area also accompanies tumor vascular heterogeneity. In addition, the reduction of tumor blood flow restricts the extravasation of molecules even more.[2]

If the anticancer drug reaches tumor interstitium, it must uniformly distribute through it in order to reach and destroy each tumor cell.[1-3] The transport of the molecule in the tumor interstitium is also governed by diffusion and convection, only here the diffusive and the convective flow are proportional to gradients instead of differences in concentration and pressure, respectively. Proportional constants are the diffusion coefficient and the hydraulic conductivity. One-dimensional transport by the diffusion in a medium is given by Fick's law:[4]

$$J_D = -D \times (\partial C/\partial x)$$

where: J_D is the diffusive flow of the solute per unit area normal to the surface (moles/s×m^2 or g/s×m^2); D is the diffusion coefficient of the solute in the medium (m^2/s), and $\partial C/\partial x$ is the concentration gradient of solute (moles/m^4 or g/m^4) in x direction. Similary, the convective flow is given by:[4]

$$J_C = -C \times R_F \times K \times (\partial p/\partial x)$$

where: J_C is the convective flow of solute per unit area normal to the surface (moles/s×m^2 or g/s×m^2); R_F is the retardation factor (solute convective velocity/solvent convective velocity); C is the concentration of solute (moles/m^3 or g/m^3); K is the tissue hydraulic conductivity (m^2/Pa×s); and $\partial p/\partial x$ is the hydraulic pressure gradient (Pa/m) in x direction.

Their values in tumor are higher than in normal tissue, but the problem of the heterogeneous distribution of anticancer drug in tumor remains. Theoretical and practical researches of the IFP profile in solid tumor revealed that it is uniformly high over the center of the tumor and that it drops rapidly at the periphery of the tumor.[5,10-12] Unfortunately the convective interstitial fluid flux is created due to pressure gradient at periphery of the tumor. Considering that the time a large molecule needs to travel some distance by diffusion is proportional to the square of the distance and the time for a movement by convection is proportional to the distance alone, it is obvious that macromolecule that was washed out of the tumor with the convective flux has a hard time diffusing back into the tumor.[3,4]

Shortly said, due to heterogeneous tumor vasculature, the increased vascular permeability and hydraulic conductivity, elevated IFP, and IFP gradient in tumor very little drug gets in the tumor. Furthermore it is washed out rapidly.

Measurement of interstitial fluid pressure

Measurement techniques for IFP measuring are numerous, but a provocative question appears: which one is referential? Although a method seems to be reliable, accurate and repeatable, it is not necessary that it measures true IFP value. In 1971 Guyton *et al.* presented an extended study on IFP.[13] They classified tissue pressure into three categories: solid tissue pressure, IFP, and total tissue pressure, which is the sum of first two. Physiological structures that cause solid tissue pressure are two: I. solid elements in the tissue interstitium such as collagen, elastin, and other types of fibers; and II. interstitial gel composed primary by mucopolysaccharides possibly or probably cross-linked with collagen. IFP

on the other hand is the pressure of the free fluid in the tissue interstitium.[13] Nevertheless, other authors called interstitial free fluid phase an abstraction since interstitium may completely consist of a gel.[14] Guyton *et al.* also critically reviewed a number of measuring techniques which they divided into two groups: needle or capillary pipette techniques and fluid equilibration techniques.[13] They expose one of the fluid equilibration techniques (perforated-capsule method) as accurate and reliable technique for IFP measuring. In order to get IFP value with this technique a small hollow plastic sphere with little holes must be implanted into the tissue. It should be implanted for weeks so that interstitial fluid can fill it and creates pressure equilibrium. A needle connected to a pressure-measuring device is then inserted in the sphere through one of the holes.[13] However, this method does not enable instant measurements, which is important, when IFP in solid tumor is measured. Therefore, currently only two measuring methods, wick-in-needle and micropuncture techniques, are used in tumor IFP measurement. In this paper we describe both of them.

Wick-in-needle (WIN) technique

The method was first presented by Fadnes *et al.* in 1977.[15] They took benefits of needle and wick techniques and combined it into one. The main problem with the needle technique is the obstruction of needle tip. They solved it by creating a 2-4 mm sidehole cca. 5 mm from the tip of the 0.6mm hypodermic needle. The needle is then filled with strand of about 25 mm thick nylon fibers. The hole of the needle tip is not closed, yet densely filling of the wick, there represented a relative larger resistance to fluid comparing to the sidehole. Therefore, the sidehole has the role of the interface area. By inserting wick into the needle they reduced trauma that a fairly thick cannula, holding the wick in the wick technique, created to the tissue. A wick-filled needle is then connected to a pressure transducer via polyethylene tube and filled with physiologic saline. The whole system is air-proof so that tissue pressure is conducted through water column to the transducer where it is recorded. The calibration is performed by placing the needle in a saline-filled beaker, or in a saline drop, placed at the level of needle insertion. The method is simple and convenient way of measuring IFP. Pressure measurements are also reproducible and stable.[15] Compared to micropuncture method WIN technique provided comparable results when s.c. and hindlimb muscle IFP in rats were measured.[14] The major problem with WIN method is that it is still traumatic (23-26G needles) to the tissue and may change local IFP due to alterations in capillary pressure, permeability and effective surface area.[15] An effect of colloid osmotic pressure to IFP measurements with WIN technique should also be considered. The osmotic flow of fluid out of the wick, due to concentration difference of proteins between interstitial fluid and physiologic saline in wick, can lower the value of the measured pressure. However, Fadnes *et al.* proposed that small fluid volume contained in the wick prevents this artifact.[15] To minimize osmotic influence furthermore, heparinized (70 units / ml) physiologic saline can be used instead of pure physiologic saline.[8,16-21] In tumor IFP measurement the accurate position or depth (e.g. in steps of 0.1 mm) of the interface spot of the needle can not be determined, however, the evaluation of needle position in tumor can be done.[10]

Micropuncture (MP) technique

The method was first used by Wiederhielm *et al.* in 1964.[14] Sharpened glass capillary (micropipette) with tip diameter of 1-4 mm is connected to a servocontrolled counterpressure device. A micropipette is filed with 0.5M NaCl solution colored with Evans blue dye.[14] As in WIN technique, the water column is used to measure the pressure exerted by the interstitial fluid to pipette fluid. Servocontrolled counterpressure system responds to the changes in the electrical resistance in the pipette tip. The increasing of the fluid pressure surrounding the pipette tip causes the tendency of this fluid to enter the pipette. Therefore, the resistance increases and the servosystem applies a counterpressure to obtain the preset resistance (equilibrium of fluids).[14] Since micropipettes are very fragile, a micromanipulator is needed to maneuver it. This also requires the immobilization of the tissue, usually implying general anesthesia and that insertion of micropipette into the tissue is performed under a guidance of a microscope.[14] The calibration of MP method is the same as in WIN technique but can also be done in a saline test chamber.[5] Guyton *et al.* in their study characterized MP technique as a method which is only capable of measuring total tissue pressure since it needs larger free-fluid spaces that are those in usual normal tissue.[13] Later Wiig *et al.* suggested that the requirement for IFP measurement with MP

technique is not free fluid but the possibility of moving fractions of nanoliters of fluid into or out of the pipette.[14] They also proposed that the MP technique is the most reliable method for measuring IFP available today. Due to guidance of micropipette with micromanipulator very accurate positions (depths) of measurement points could be determined. Disadvantages of the method, however, are fragility of the micropipettes, urgent immobilization of the tissue, limited insertion range compared to WIN technique, and that the stretching or the compressing of the tissue, due to micropipette withdrawal or insertion, causes lower of higher pressures, respectively.[14]

Changes in IFP due to different treatment approaches

In 1950 Young *et al.* first demonstrated that IFP in solid tumors is increased.[22] Since it is known that increased IFP impedes the antitumor treatment many studies were performed to reduce it.

Hyperthermia

Hyperthermia is a cancer treatment where tumor is exposed to overheating with temperature up to 42-45°C or higher.[23] In their research Leunig *et al.* found out that local hyperthermia at 43°C for 60 min reduces IFP value in amelanotic melanoma A-Mel-3 from 1675.8 Pa to 106.4 Pa.[24] They assumed that the main reason for lowering IFP was the reduction of a local MVP to zero due to impaired tumor microvasculature. Their theory was that at the beginning of hyperthermia blood flow in tumor may increase, thus bringing more drug to the tumor. Later, when the reduction of blood flow begins, drug remains in tumor. Therefore, decreased IFP may increase the delivery of the drug to the tumor by facilitating the extravasation and by reducing the washing out of the drug.[24] On the other hand, Hauck *et al.* treated D-54MG glioma xenograft with the exposure to 41.8°C for 4h. They observed no changes in IFP after the tumor treatment.[25] Inconsistency of both studies could be a result of different treatment protocoles as well as different tumor models. Nevertheless, further studies are needed to determine whether hyperthermia has an effect on IFP.

Radiation

Radiation is one of the conventional antitumor treatments,[1] but the hypoxic nature of the tumor usually impedes the radiation effectiveness.[3,16] In this field of interest the question appears, how does the radiation affect IFP. Znati *et al.* investigated the effect of radiation on the IFP and determined the minimum dose required to affect IFP.[16] They used xenografts of LS174T human colon adenocarcinoma. The radiation dose below 10 Gy (2×2.5 Gy) did not significantly change IFP, whereas doses of 10 Gy and above (2×5 Gy and 2×10 Gy) decreased IFP value for 332.5 Pa (initial value was 1715.7 Pa). They also found out that the maximum reduction of IFP (35%) after the single-dose irradiation appeared 5 days after the radiation with 30 Gy and then started to increase again. The radiation of 10 and 20 Gy reduced IFP for 19 and 23% respectively, in both cases 3 days after the irradiation. They assumed that the observed decrease in IFP was the result of the reduction of microvascular pressure, due to the decreased venous vascular resistance. Roh *et al.* on the other hand measured IFP in carcinoma of the uterine cervix *in situ* after the irradiation.[18] They found out very high values of IFP (maximum value 5453 Pa) which exceeds that in many experimental tumors. They also measured IFP in normal uterine cervix and found it to be between 0 and 399 Pa. After the fractionated irradiation from 32 to 60 Gy they observed the decrease in IFP in 4 patients, whereas in 3 patients IFP remained unchanged or increased. The mechanism that causes the IFP reduction in some tumors and not in others remains unknown. They suggested however, that the correlation between changes in IFP and the tumor response to therapy, which they observed, could be useful in predicting a treatment outcome and in determining future strategies for treatment.[18]

Vasoactive agents

Based on a hypothesis that local MVP governs IFP, we can conclude that changes in MVP should result in IFP changes. To decrease (or increase) MVP two different approaches may be used: decrease (or increase) in mean arterial blood pressure (MABP) and increase (or decrease) in tumor venous resistance.[21] Both physiological factors can be manipulated with drugs that have the effect on vasculature (vasoactive drugs). Lee *et el.* studied the effects of the pentoxifylline (PTX) on IFP in FSaII murine tumors.[21] They found out 55% decrease in IFP which

reached its minimum 2 hours and remained up to 4 hours at this level after the injection of 100 mg/kg PTX. They suggested that the observed reduction of the IFP was due to decrease in the tumor venous resistance as described in the above hypothesis. In a similar comprehensive study Zlotecki *et al.* observed changes in IFP due to five different vasoactive drugs and compared it with mathematical model.[26] Three vasoconstricting agents angiotensin II, epinephrine, and norepinephrine increased IFP as well as MABP. However, the magnitude of increase in IFP (ΔIFP and IFP ratio post/pre) was significantly different for each agent whereas the increase in MABP was similar. Angiotensin II increased IFP for 838 Pa, epinephrine increased it for 386 Pa and norepinephrine for 186 Pa. Initial values were between 2394 and 2926 Pa. Two vasodilating agents (hydralazine and nitroglycerin) decreased IFP and MABP. Hydralazine which is a long-acting vasodilator causes 50% decrease in IFP (2261 Pa) over the first hour and nitroglycerin which is a short-acting vasodilator produced only small, transient 6% (173 Pa) decrease in IFP. Initial values of IFP before the hydralazine injection was 4389 Pa and before the nitroglycerin injection 2886 Pa. These results correlate with changes in MABP. In their study they also analysed the experimental results with a mathematical model. Briefly, they found out that changes in IFP are mainly due to the effect of vasoactive agents on tumor or on the surrounding tissue vasculature resistance.[26] In a similar study Tveit *et al.* measured IFP after the administration of norepinephrine. In contrast to the previous study of Zlotecki *et al.* they found out that IFP decreased form initial value of 1370 Pa to 931 Pa. This inconsistency of their results with results of Zlotecki *et al.* was not explained.[26]

In addition, other studies determining the effects of hemodilution and TNF-α on IFP were also performed.[20,27] In both of these studies authors report the decrease of IFP.

Conclusions

The elevated IFP together with the heterogeneous blood supply in solid tumors are the reasons for the inadequate uptake and nonuniform distribution of the anticancer drugs (especially macromolecules).[18] The main reasons for the increase in IFP are high vascular permeability and hydraulic conductivity, high tumor vascular resistance to blood flow, and lack of functioning lymphatic system.[16] It was demonstrated that IFP is mainly governed by the MVP which is also increased, due to high tumor venous resistance. Therefore, MVP values in tumors are the same as IFP values.[8] IFP in tumours is uniformly elevated throughout the tumor but drops rapidly in the periphery of the tissue-isolated tumors and at the tumor-normal tissue interface in tumors surrounded by normal tissue.[2,3,5,12] This property causes fluid to ooze out of tumor and thus create radial convection of macromolecules which are located into the tumor.[2,8,16,18]

Measurement techniques currently used to measure IFP in tumors are two: WIN technique and MP technique. Although MP technique is controlled by the counterpressure system and is though more accurate, WIN technique is, in spite of its simplicity, quite adequate to produce reliable and accurate results. Both techniques however, have their benefits and weaknesses.

Many methods were used to reduce IFP in tumors and thus optimize the anticancer drug uptake but none of them brought satisfactory results. Nevertheless, elevated IFP would present very few or no problems for the antitumor treatment with a molecule or cell which has nearly 100% specificity for tumor cells.[2] Meanwhile, novel treatment approaches must be found out that will eliminate these physiological barriers and will thus enable antitumor agents to complete their mission.[2] Only a complex antitumor treatment which will affect all carcinogenic mechanisms at the same time will be a successful one. Better understanding in IFP represents a step towards the effective antitumor treatment.

Appendix

All pressure units in this article are in SI system (Pascal - [Pa]) although pressure units used in references are in mmHg. Therefore we used transformation equation:[28]

$$101325 \text{ Pa} = 1 \text{ atm} = 760 \text{ mmHg}$$
$$1 \text{ mmHg} \cong 133 \text{ Pa}$$

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