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# Dosage Estimation Of Amino-Modified Polystyrene-Antisense Human RNA Nanoparticles In Killing Metastasis Based On The Tumor Growth Rate

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***Abstract*—Amino-modified polystyrene-antisense human RNA nanoparticles ($NH_2$PS-antisense hTR) kill metastatic cells that expresses telomerase RNA in cytoplasm [(1)]. The dosage of less than 100 ng/ml blood level is recommended to cause cell arrest at G2 phase in the culture plate[(2)]. Higher dose causes tumor lysis syndrome and liver damage due to cytochrome P450 reduction.**

**Problem arises as the tumor cells can duplicate again 24-48 hours after stopping the nanoparticle administration**. Moreover, some tumors like sarcoma grow at a faster rate, the metastasis will kill patient despite 100 ng/ml blood concentration of this nanoparticle. Patient will die when the lethal tumor volume ($10^{12}$ cells) is reached as described by Norton[(3)].

In-vivo dosage of nanoparticles should be more than 100 ng/ml blood level because $NH_2$PS-antisense hTR may not be fully dissociated to form $NH_2$PS, and there is sequestration by mononuclear phagocytic system (MPS) in various organs, metabolism by hepatic cytochrome P450 enzyme, and excretion by our kidneys.

By mathematical deduction, the in-vivo blood level is slightly less than 156.7ng/ml. The dosage and dosing interval can be worked out by pharmacological formula.

Fabrication and modification of surface charge of nanoparticle affects the organ distribution, making it more efficient in treating different metastatic organs[(4)].

***Keywords*—*Amino-modified polystyrene-antisense human RNA nanoparticles, exponential tumor growth rate, tumor doubling time, cytochrome P450, renal clearance, blood flow rate of tissue, steady state plasma nanoparticle concentration***

## I. INTRODUCTION

On intravenous administration, nanoparticles (NPs) enter the blood stream, undergo protein adsorption or opsonization to form a larger hydrodynamic diameter (HD). The particle size is inversely proportional to its glomerular filtration rate, and directly proportional to the serum half-life.

The pore sizes of vascular endothelium and the lymphatic endothelium are 5nm and 6 nm respectively. Modification of the size, shape, surface chemistry, e.g. by attaching polyethylene glycols to surface alters the half-life, distribution, and elimination of the NPs.

Tumor, that once develops despite body's surveillance system, will grow exponentially and locally at first. Surgical excision is curative at this stage. As time goes by, this tumor will grow bigger, and finally metastasize. **Chemo- and radio-therapy may not be able to kill the metastasis due to the defensive mechanisms developed by tumor cell.**

However, the mRNA of the essential protein, like telomerase, cannot be changed for the survival of the species. By attaching the $NH_2$PS-antisense hTR to the cytoplasmic mRNA of telomerase, $NH_2$-PS will be released and the tumor cell growth will be arrested in G2 phase[(2)].

**This NP can cause liver damage because hepatic cytochrome P450 can also reduce it to $NH_2$-PS.** The solution is to block the corresponding cytochromes.

To make dosage estimation possible, basic physiological, and pharmacological principles are used.

## II. METHOD OF DOSAGE ESTIMATION

The initial exponential growth rate of tumor will decrease gradually due to the limitation of nutrients, oxygen, and the accumulation of metabolic waste including the oxygen radicals.

To achieve an optimal tumor growth, the oxygen radicals need to be removed by reducing agent. So tumor cell, just like normal cell, grows better in the presence of reducing agents, like vitamins A, C, E or natural reducing agents like blue berries. Just before the establishment of malignancy, does the healthy drugs or food (containing the reducing agents) be protective?

Initial exponential tumor growth (dN / dt) depends on the existing number of tumor cells(N), and the tumor

growth rate (r)

$dN / dt = r N$ (1)

Integrating both sides from $t_0$ to t.
At $t_0$, time is 0 and N is N(0). At time t, N is N(t).

$\int (1/N)\, dN = \int r\, dt$
$\ln N(t) - \ln N(0) = r t - r t_0$
$\ln [N(t) / N(0)] = r (t - t_0)$

Anti-log over both sides and rearrange
$N(t) = N(0) \times e^{rt}$ (2)

**Tumor doubling time** ($T_d$) means the time when N(t) = 2 N(0). From equation (2), we have $T_d = (\ln 2)/r$ .

We measure the tumor volume $q_1$ and $q_2$ at 2 time points $t_1$ and $t_2$.

$q_1 = q_0 \times e^{rt1}$
$q_2 = q_0 \times e^{rt2}$
$\ln(q_2/ q_1) = r (t_2 - t_1)$
$r = \ln(q_2/ q_1) /(t_2 - t_1)$ (3)
$T_d = (\ln 2)(t_2 - t_1)/ \ln(q_2/ q_1)$ (4)

By measuring the area of the tumor a cut in the CT imaging, the tumor volume can be calculated by the formula :

$\Delta \text{Area}^{3/2} = \Delta \text{Volume}$

In general, the tumor growth rate is governed by the power-law differential equation[4]

$$\frac{dV}{dt} = rV(t)^{\alpha} \quad (5)$$

When α = 1 this reduces to equation (1).

When α < 1 , the solution is

$$V(t) = (V_0^{1-\alpha} + (1-\alpha)rt)^{1/(1-\alpha)} \quad (6)$$

Due to the shortage of nutrients or chemo-radio-therapy, a portion of tumor cell will die. The power-law with linear death has the form

$$\frac{dV}{dt} = rV(t)^{\alpha} - r\frac{V(t)}{K^{1-\alpha}} = rV^{\alpha}\left(1 - \left(\frac{V}{K}\right)^{1-\alpha}\right) \quad (7)$$

The growth rate changes to exponential again once the tumor metastasizes to a new clean site, with adequate nutrition and lack of metabolic wastes.

Intravenous $NH_2$PS-antisense hTR nanoparticles kills a certain fraction of tumor. Let r be the tumor growth rate and $r_k$ be the tumor killing rate of $NH_2$PS-antisense hTR in vitro.

Equation 1 becomes $dN / dt = (r - r_k) N$ (8)

In our bodies, killing rate ($r_k$) of this NP is less because part of this nanoparticle will be metabolized by hepatic cytochrome P450 system.

## III. Hepatic Metabolism of Nanoparticle

In liver, particulates undergo catabolism in hepatocytes, biliary excretion and elimination. Kupffer cells (part of MPS) possess receptors for selective endocytosis of opsonized particles. NPs molecular modification via PEGylation will decrease first-pass metabolism and increase the serum half-life.

Let $r_h$ be the rate of hepatic metabolism.
$r_h = CL_{int} \times C_E$ (9)
where $CL_{int}$ is the hepatic clearance and $C_E$ is the free drug concentration at the hepatic enzyme site .

$r_h$ can be derived from the Michaelis–Menten equation

$$v = \frac{d[P]}{dt} = \frac{V_{\max}[S]}{K_M + [S]} \quad (10)$$

The rate of enzymatic reaction (v) is related to the substrate concentration [S].

$$v = \frac{d[P]}{dt} = \frac{V_{\max}[S]}{K_M + [S]} \quad (11)$$

where $V_{max}$ is the maximum rate achieved by the hepatic cytochrome P450 system, at saturating nanoparticle concentration, and $K_m$ is the Michaelis constant and equals substrate concentration [S] at half $V_{max}$ .

Normally the free drug concentration $C_E$ is ≤10% of the Michaelis constant $K_m$ . Put $C_E$ be [S] and approximate ($K_m$ +[S]) to $K_m$, we have $r_h = v = V_{max} \times C_E / K_m$ (12)

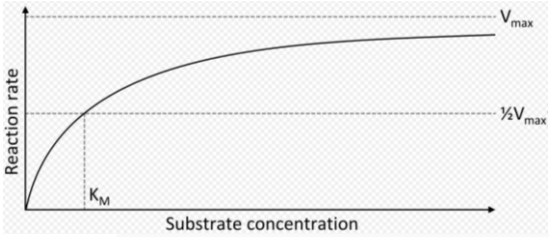


Figure 1. Michaelis–Menten saturation curve for an enzyme reaction showing the relation between the substrate concentration and reaction rate [5]

So $dN / dt = [\, r - (r_k - r_h)\, ] N$ (13)

## IV. Reduce the Hepatic Toxicity of This Nanoparticle

For amine reduction, liver microsomal enzymes cytochrome 3A4, 3A5, 2C19 being the most important as shown on the metabolism of Zonisamide [7].

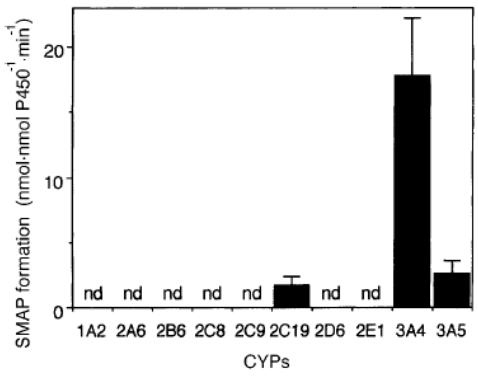


Figure 2. Metabolism of Zonisamide by expressed human cytochromes [7].

| | $k_M$ ($\mu mol \cdot l^{-1}$) | $V_{max}$ ($nmol \cdot nmol\ P450^{-1} \cdot min^{-1}$) | $V_{max}/k_M$ |
|---|---|---|---|
| CYP2C19 | 188 | 3.6 | 0.019 |
| CYP3A4 | 247 | 33.0 | 0.134 |
| CYP3A5 | 179 | 4.7 | 0.026 |
| Human liver microsomes | 274 | 1.5 | 0.0055 |

Table 1. Kinetic parameters of metabolite in lysates of cell expressed cytochromes and human liver microsomes [7].

According to the zonisamide study, hepatic CYP3A4 is the most important enzyme for reduction. We can use CYP3A4 inhibitors, like clarithromycin, erythromycin, diltiazem, verapamil, grapefruit juice, itraconazole, ketoconazole, ritonavir to reduce the liver toxicity.

In case of **CYP3A4 irreversible inhibitor (e.g. clarithromycin)**, this nanoparticle clearance is much reduced, whereas in **competitive inhibition (e.g. ketoconazole)**, the metabolic clearance is expressed by

$$CL_{int} = V_{max} / [\, k_M (1 + I_u / K_i) + C_u \,] \qquad (14)$$

where $k_M$ is Michaelis constant of amine nanoparticles metabolism, $V_{max}$ is the maximum metabolic rate of amine nanoparticles, $C_u$, $I_u$ are unbound amine nanoparticles and unbound ketoconazole concentration, $K_i$ is the inhibition constant of ketoconazole metabolism, C is the amine nanoparticles concentration[7].

Since $k_M$ is >> $C_u$ , the equation becomes

$$CL_{int} = V_{max} / [\, k_M (1 + I_u / K_i) \,] \qquad (16)$$

However, if amine nanoparticle is overdose, $C_u = a\ k_M$ where a >1 . The equation becomes

$$CL_{int} = V_{max} / [\, k_M (a + 1 + I_u / K_i) \,] < V_{max} / [\, k_M (1 + I_u / K_i) \,] \qquad (17)$$

So clearance of amine nanoparticles is slower in case of drug overdose even if we give ketoconazole to patient[6].

## V. RENAL EXCRETION OF AMINE NANOPARTICLES

As the nanoparticles enter the glomerular capillary bed, they are filtered through the fenestrated endothelium, glomerular basement membrane, and the foot processes of glomerular epithelial cells. The functional pore size of glomerular filtration is 4.5-5.0 nm.

Charged nanoparticles undergo protein adsorption and interact with charges within the glomerular capillary wall as shown in quantum dots model. PEGylation and zwitterionic coatings prevent the protein adsorption.

At the proximal renal tubule, the filtered molecules like glucose will be resorbed. The unfiltered molecules like heavy metals will actively secreted into the tubular lumen and cause damage.

Let $r_r$ be the renal excretion rate of $NH_2$PS-antisense hTR nanoparticles. It depends on the renal clearance and the nanoparticle mass in kidney at time *t*

$$d\,M_{urine} / dt = CL_{kidney}\, M_{kidney} \qquad (18)$$

where $M_{urine}$ is the accumulated nanoparticle mass in the urine, $CL_{kidney}$ is the excretion coefficient for the kidney, and $M_{kidney}$ is the nanoparticle mass in kidney at time *t*.

The upper limits of diffusion coefficients were determined based on blood flow rates. **It could not be faster than the rate at which blood carries nanoparticles into that tissue.**

$\mathbf{K_i \le Q_i / V_p}$ where $Q_i$ is the blood flow rate of tissue *i* and $V_p$ is the volume of plasma.

$$r_r = dM_{urine} / dt \le Q_r / V_p \qquad (19)$$

$$dN / dt = [r - (r_k - r_h - r_r)]\, N \qquad (20)$$

## VI. OPSONIZATION / SEQUESTRATION BY THE MONONUCLEAR PHAGOCYTE SYSTEM (MPS)

MPS in liver, spleen and lungs sequesters nanoparticles. Serum proteins are adsorpted on the surface of NPs to form corona. (PEGylation of NPs hinders the protein adsorption.) The corona attaches to the surface receptors, being internalized, transported to phagosomes, and fused with lysosomes inside the macrophage . $NH_2$PS nanoparticles are nontoxic to macrophages because the latter do not rely on the mTOR (mammalian target of rapamycin) signaling for survival.

Let the rate of sequestration in liver, lungs, spleen, and GI tract be $r_h$ , $r_l$ , $r_s$ and $r_{GI}$ , which are less than or equals to $Q_h / V_p$ , $Q_l / V_p$ , $Q_s / V_p$ and $Q_{GI} / V_p$ . The equation can be refined as

$dN/dt = (r + r_h + r_r + r_l + r_s + r_{GI} - r_k) N$

$dN/dt = [\ln(q_2/ q_1) /(t_2 - t_1) + (Q_h + Q_s + Q_{GI} + Q_r + Q_l)/ V_p - r_k] N$ (21)

(where killing rate $r_k$ can be measured in the tissue culture plate in vitro and the organ perfusion *Q of liver, kidneys, lung, spleen* can be measured by perfusion scan.)

| Organ | % of cardiac output |
|---|---|
| Lung | 100% |
| (Bronchial arterial flow) ∫ | (0.6%) |
| Liver | 27.0% |
| Kidneys | 23.3 % |
| Spleen ∩ | 5.0% |
| Brain | 13.9 % |
| Skin | 8.6 % |
| Skeletal muscle | 15.6 % |
| Heart muscle | 4.7 % |
| Rest of body | 6.2 % |

Table 2.The percentage of cardiac output to different organs. ∫ Measurement of bronchial blood flow in the sheep by video dilution technique. Thorax 1985;40: 143-149. ∩ http://www.ihaematology. com/general-haematology/splenic-physiology

Splenic sequestration of nanoparticles should be less than 5% of the serum nanoparticle loading. So $Q_s/ V_p < 0.05$

Liver receives blood from hepatic artery (6%) and portal vein (21%) accounting for 27% of cardiac output. Liver sequestration should be less than 27% of the serum nanoparticle loading. So $r_h + r_{GI} = (Q_h + Q_{GI})/ V_p < 0.27$

As kidney receives 22% of cardiac output and so $r_r = Q_r / V_p < 0.22$

In experimental models, lung sequestration of NPs varies from 2-10% of liver sequestration. So $Q_l / V_p < 0.027$

Pulmonary uptake of NPs depends on the size and surface charge.

Intravenous NPs administration precludes the enzymatic degradation at the mucosal surfaces of respiratory tract and the gastrointestinal tract.

In tumor tissue, there is enhanced permeability and retention effect. NPs with size 100–200 nm can extravasate through tumor vascular fenestrations into the tumor cells, enhancing the tumoricidal effect.

Integrates both sides of equation (21)

$N(t)=N(0)e^{(\ln(q2/ q1) /(t2 - t1) +0.27 + 0.05+ 0.22+0.027- r k)t}$

$= N(0)e^{(\ln(q2/ q1) /(t2 - t1) +0.567- r k)t}$ (22)

To keep patient alive, $N(t) < 10^{12}$ cells. To shrink the metastasis, the net tumor growth rate should be less than zero. So

$\ln(q2/ q1) /(t2 - t1) +0.567- r_k < 0$

$r_k > \ln(q2/ q1) /(t2 - t1) +0.567$ (23)

In the culture plate, we use 100ng/ml to arrest tumor growth at G2 phase. For each ml of cardiac output, 0.567ml extra volume of NPs is required to fill up the sequestration by the macrophages

To make the blood NPs concentration the same, we should have 156.7 ng/ml blood level.

Steady plasma nanoparticle concentration $\overline{C}p_{ss}$ is kept to be 100ng/ml by the following formula.

$$\overline{C}p_{ss} = \frac{D}{CL \cdot \tau}$$ (24)

where D = the dose of nanoparticle given intravenously, $\tau$ = dosing interval and CL = hepatic + renal clearance (nanoparticles are sequestrated in spleen and lung only, but metabolized and excreted in liver and kidney)

If the given dose is smaller, the dosing interval should be shorter in order to achieve a steady plasma nanoparticle concentration.

To reduce the drug toxicity, we can change IV drug bolus to continuous IV drug infusion using a smaller dose per unit time.

## VII. FABRICATION AND CHARGE-MODIFICATION TO AFFECT THE ORGAN DISTRIBUTION OF NANOPARTICLES

Interesting, fabrication of nanoparticles affects the organ distribution of NPs [(4)].
Surface charge of NPs influences the opsonization, and the circulation times. With positive charge, NPs are more prone to sequestration by macrophages in the lungs, liver and spleen. Neutral or negatively charged NPs have longer circulation time and less accumulation in the above organs[(4)] .

To reduce liver toxicity, NPs with diameter less than 5nm, neutral / negatively charged are used. To enhance renal excretion, NPs with size less than 5nm are used, although the **feasibility of making a so-small nanoparticle is a question.**

To treat the liver or the lung secondary, we use NPs with diameter more than 150nm, discoidal shape, and with positive surface charge.

In leukaemia of lymphoma involving liver and spleen,

we use NPs with size more than 150nm, discoidal or cylindrical shape, and positive surface charge.

It is better to verify the theoretical dosage, and observe the tumor response in the long run in rat model before clinical trial on patients.

Conclusion and discussion:

$NH_2Si$ (amino-silica) nanoparticle carries antisense oligonucleotide to interfere with the telomerase gene expression at the mRNA level in cytoplasm[8].

Due to the structural similarity, $NH_2$-PS (amino-modified polystyrene) can replace $NH_2Si$ to deliver antisense oligonucleotide[1].

$NH_2$-PS, once released, is cytotoxic and kill the metastatic cells. Inevitably, some normal stem cells and gonadal cells are also killed as they also contain telomerase, although to a lesser extent.

Hepatic cytochrome P450 can also reduce the $NH_2$-PS-antisense oligonucleotide to release $NH_2$-PS. Cytochrome P450 inhibitor can reduce the liver toxicity without affecting the tumoricidal effect of the NPs.

Studies has shown that NPs sequestration is due to the resident macrophages mainly in liver, spleen, and lung. The sequestration cannot be greater than the percentage blood flow to that organ.

Due to the portal venous blood flow, the hepatic clearance of NPs includes that of gastrointestinal tract. Dosage estimation can be simplified by simple arithmetics of the tumor growth rate, renal excretion rate, organ sequestration rate, and the tumor killing rate.

References:

1. Szeto CH. Review on telomerase activity in metastasis and proposal of amino-modified polystyrene-antisense human RNA nanoparticles in killing metastatic cells. *Mediterr.j.bio., 1(3), 2016.*

2. Kim, Jong Ah. Low Dose of Amino-Modified Nanoparticles Induces Cell Cycle Arrest. ACS NANO, 7 (9): 7483-7494.

3. Norton, L. (1988) A Gompertzian model of human breast cancer growth. Cancer Research. 48, 7067–7071

4. Elvin Blanco. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nature Biotechnology 33, 941–951 (2015)

5. Anne Talkington. Estimating tumor growth rates in vivo. Duke University Mathematics Department. November 19, 2014.

6. Michaelis-Menten Kinetics. Wikipedia

7. Nakasa H. Prediction of drug-drug interactions of zonisamide metabolism in humans from in vitro data.Eur J Clin Pharmacol. 1998 Apr;54(2):177-83.

8. Peng J. An antisense oligonucleotide carrier based on amino silica nanoparticles for antisense inhibition of cancer cells. Nanomedicine 2006 Jun;2(2):113-20.