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A Pharmacokinetic Simulation Model for Chemotherapy of Brain Tumor with an Antitumor Protein Antibiotic, Neocarzinostatin

Theoretical Considerations Behind a Two-Compartment Model for Continuous Infusion via an Internal Carotid Artery

Hiroshi Maeda[1], Yoshinori Sano[2], Jiro Takeshita[1], Zenta Iwai[3], Hideyuki Kosaka[2], Tohru Marubayashi[2], and Yasuhiko Matsukado[2]

[1] Departments of Microbiology and
[2] Neurosurgery, Kumamoto University School of Medicine, Honjo 2-2, Kumamoto
[3] Department of Mechanical Engineering, Faculty of Engineering, Kumamoto University, Kurokami 1-1, Kumamoto, Japan 860

Summary. *A pharmacokinetic two-compartment model for the treatment of brain tumors in man was simulated with the aid of a computer. The parameters necessary for the simulations such as inactivation rate constant, elimination rate constant, distribution volume, blood volume, cerebral blood flow, and cytotoxic drug concentration were either determined in this study or obtained from the literature. A proteinaceous antitumor antibiotic, neocarzinostatin (NCS), was utilized as a prototype drug because it has features making it advantageous in the treatment of brain tumor. In particular, NCS has an extremely short half-life in serum ($t_{1/2} \leq 3$ s), while it is relatively stable in the cerebrospinal fluid (CSF) ($t_{1/2} \sim 50$ s). Therefore, the drug level in the cerebral compartment can be made adequately high with an appropriate infusion velocity into the cerebral compartment; however, it was possible to keep the plasma level of the drug much lower than the toxic level. Thus, few side-effects should result. In an in vitro study, NCS was found to exhibit its cytotoxicity to glioblastoma cells at a concentration as low as 0.005 μg/ml. In contrast, the cytotoxicity was not apparent for the normal glia cells at 0.1 μg/ml. The model being considered in this investigation is a two-compartment model, which consists of the cerebral compartment and the rest of the circulatory system of the body. In this case the drug is infused via an internal carotid artery. The results of pharmacokinetic simulation and dose regimens for NCS are presented, based on the effective concentration of the drug to glioblastoma cells in culture and the available pharmacological parameters.*

Reprint requests should be addressed to: H. Maeda

Introduction

Brain tumors are most frequently treated by surgery and radiation. However, these methods are limited to the localized area of tumors, while the invisible tumor micrometastasis spreads, frequently to the cerebrospinal compartment. Therefore, chemotherapy is an alternative choice despite its drawbacks, such as non-selective toxicity to CNS (central nervous system) or hematopoietic and hepatic organs. With this in mind we have initiated chemotherapy with a proteinaceous antitumor antibiotic neocarzinostatin (NCS) [5, 11], which appears to exhibit a strong cytotoxicity against glioblastoma cells as described in this report. NCS is a small simple protein with a molecular weight of 11,000 and is well characterized chemically and biologically [6, 7, 12, 19]. Clinical efficacy has been established in leukemia, lymphoma, and bladder and other cancers in man. The effect of NCS on brain tumors, however, has yet to be established. It is unique in that it is readily degraded by various proteases in serum and by cells, resulting in an extremely short in vivo half life ($t_{1/2}$), about 5 s at at 0.1 μg/ml in serum [15]. In contrast, its $t_{1/2}$ in CSF is about 16 times longer than that in serum. NCS also possesses a rapid renal clearance rate ($t_{1/2}$ about 7 min) [13, 14]. This rapid renal clearance rate is a pronounced contrast to other drugs of low molecular

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weight, most of which have a $t_{1/2}$ of 1–4 h. This indicates that NCS, which has a molecular weight of 11,000 daltons, behaves in a similar way to some of the low-molecular-weight substances. It is also known that NCS leaks out of blood capillaries readily and is recovered effectively in the lymphatic system [16]. Another unique feature of NCS is its high sensitivity against glioblastoma cells: the minimum effective concentration against glioblastoma cells in culture, is less than 0.005 μg/ml, as described below, whereas NCS is not toxic against non-dividing cells at 0.1 μg/ml.

Maeda et al. reported that NCS did not cross the blood-brain barrier [13]. This indicates that the CNS toxicity of NCS is low when the drug is given systemically. However, the blood-brain barrier appears to deteriorate when the malignant tumor is in the brain [9]. Therefore, NCS infused via an artery will leak out of the barrier and reach the site of tumors more or less selectively. This fact prompted us to devise a compartment model for the infusion of NCS, based on the fact of the extremely short $t_{1/2}$ in blood circulation in contrast to that in CSF; in this model one compartment represents primarily the cerebral blood circulation and the other, the rest of the systemic blood circulation. Four main factors support this model: Firstly, in case of a brain tumor, the blood-brain barrier has deteriorated and such a drug as NCS will leak out of capillary circulation to the CSF, which is often in contact with tumor cells. Secondly, the cerebrospinal system itself is a single compartment with little exchange with the circulating blood, so that a long $t_{1/2}$ of NCS is expected when NCS reaches this site. Thirdly, glioblastoma and rapidly growing tumor cells are much more susceptible than normal cells to NCS, according to this and other studies [10, 20]. Fourthly, at the minimum inhibitory concentration (MIC) of glioblastoma cells, no side-effects affecting the CNS or systemic side-effects will result. In view of these factors we determined and described most of the parameters necessary for such pharmacokinetic simulation of the treatment of human brain tumor. (See [3] and [4] for pharmacokinetic models).

Materials, Methods, and Theory

Drug. NCS was obtained from Kayaku Antibiotic Research Laboratories, Tokyo. NCS is supplied in dark ampoules containing 2 mg dissolved in 2 ml saline.

In vitro Susceptibility of Glioblastoma and Glia Cells in Culture to NCS. Three representative human glioblastoma cell strains, S-96, S-115, and S-122, and fetal human brain cells, all maintained in our laboratory, were cultured in test tubes containing RPMI-1640 medium enriched with 5% fetal calf serum (Gibco, Grand Island, NY, USA). With this serum concentration, $t_{1/2}$ values for NCS in the culture medium and in the CSF are almost the same. NCS dissolved in saline was added to the culture medium (2 ml) at 10% in volumes to give final drug concentrations between 0.005 and 0.1 μg/ml. The cells, inoculated at 4.0×10^4/ml in a stoppered test tube (1.3 × 10.5 cm), were allowed to grow at 37° C. NCS was added four times to the culture medium, 4 h after cell plating and 1, 3, and 8 days after cell inoculation, and the medium was changed every 4 other days after cell inoculation. The effect of the drug was evaluated by counting the numbers of cells.

Inactivation Rate Constant of NCS in CSF and in Serum in vitro. Samples of CSF and blood were obtained from patients with either malignant or non-malignant tumors or from healthy men in our University hospital. Inactivation of the drug by CSF was determined as follows. NCS was dissolved in CSF to give different concentrations and incubated at 37° C in the dark for various time periods, after which each aliquot was frozen in dry ice until assay. The assay was based on an antimicrobial activity of the drug against sensitive bacteria *Sarcina lutea* on a sensitivity test agar plate as described elsewhere [16]. The minimum detectable concentration was 0.005 μg NCS/ml, and a 50-μl aliquot was placed in a glass cylinder (∅10 mm). The incubation time with CSF was plotted against the remaining antibacterial activity for each drug level, and the time required for a 50% inactivation was plotted against the NCS concentration. Subsequently, $t_{1/2}$ values for corresponding NCS concentrations were plotted on a log-log scale.

Other Parameters Used in the Pharmacokinetic Analyses. Renal excretion rate constant and plasma inactivation rate constant were obtained from our previous reports [14, 15]. The apparent volume of distribution of NCS in human of 60 kg body weight was previously estimated to be about 8.56 l [15]. Blood flow in an internal carotid artery, which covers a cerebral hemisphere, CSF volume, and the cerbral blood volume of a hemisphere, are assumed to be 6 ml/s, 120, and 65 ml respectively [1, 18]. The replacement velocity of CSF in the compartment is assumed to be 0.35 ml/min (= 0.00583 ml/s) [18]. Furthermore, several other assumptions are made in the calculation as follows: The apparent distribution volume of the NCS in the brain, although it contains the brain tumor, is equivalent to the blood volume of the brain by virtue of the blood-brain barrier; the inactivation rate of NCS in tumor tissue is similar to that of serum (blood); the levels of the proteolytic enzyme and renal function in the individual subjects are within the normal range; no diffusion out of the cerebrospinal compartment will occur other than normal CSF replacement.

Pharmacokinetic Model.

Two-compartment Model Based on a Continuous Infusion via Internal Carotid Artery. Each of the two compartments under consideration represents a cerebral hemisphere, the first compartment (I) being where the target tumor resides and the rest of the body being represented by the second compartment (II). The purpose of the present investigation is to establish a minimum effective concentration in compartment (I) and to clarify that in compartment (II) is well below the subtoxic level of the drug. The diagrammatic representation is shown in Fig. 1.

When the balance of the drug in the two compartments during an infinitesimally short time period is considered the following equations are obtained:

$$\left.\begin{aligned} \frac{dX_{\mathrm{I}}}{dt} &= k_{\mathrm{II-I}} X_{\mathrm{II}} - (k_{\mathrm{I-II}} + k_{\mathrm{Ii}}) X_{\mathrm{I}} + k_s ; \\ \frac{dX_{\mathrm{II}}}{dt} &= k_{\mathrm{I-II}} X_{\mathrm{I}} - (k_{\mathrm{II-I}} + k_{\mathrm{IIi}} + k_u) X_{\mathrm{II}} ; \\ X_{\mathrm{I}}(0) &= X_{\mathrm{II}}(0) = 0 \end{aligned}\right\} \quad (1)$$

where X_I and X_{II} are the amounts of drug in compartments I and II at time t, respectively; $k_{I\text{-}II}$ and $k_{II\text{-}I}$ are the transfer rate constants from the compartment I-II and II-I, respectively; k_{Ii} and k_{IIi} are the inactivation rates in the serum in compartments I and II, the values for which closely depend on the NCS concentration in the serum; and k_s is the infusion rate constant and k_u the urinary rate constant. Further, X in eq. (1) can be replaced by C from eq. (2).

$$X = V \cdot C \tag{2}$$

where V is compartment volume (ml) and C is drug concentration. Then we have

$$\left.\begin{aligned} \frac{dC_I}{dt} &= -(k_{I\text{-}II} + k_{Ii})\, C_I + \frac{k_{I\text{-}II}\, V_{II}}{V_I} C_{II} + \frac{k_s}{V_I}\,; \\ \frac{dC_{II}}{dt} &= \frac{k_{I\text{-}II}\, V_I}{V_{II}} C_I - (k_{II\text{-}I} + k_{IIi} + k_u)\, C_{II}\,. \end{aligned}\right\} \tag{3}$$

The parameters and constants involved are summarized as follows:

V_I: blood volume in the brain; inside capacity of blood-brain barrier of a hemisphere, 65 ml;

V_{II}: apparent volume of distribution of the drug, NCS, for an adult (60 kg body weight) in compartment II, 8,560 ml − V_I = 8,495 ml;

V_{If}: blood flow in the brain/min via bilateral internal carotid artery, 720 ml/min;

$^{1/2}V_{If}$: blood flow in the brain via one of the bilateral internal carotid arteries, through which NCS is fed.

From above results, we have

$$\left.\begin{aligned} k_{I\text{-}II} &= \frac{^{1/2}V_{If}}{V_I} = 5.54/\text{min}\,; \\ k_{II\text{-}I} &= \frac{^{1/2}V_{If}}{V_{II}} = 0.042/\text{min}\,; \\ k_{II\text{-}I} \cdot \frac{V_{II}}{V_I} &= \frac{^{1/2}V_{If}}{V_I} = 5.54/\text{min}\,; \\ k_{I\text{-}II} \cdot \frac{V_I}{V_{II}} &= \frac{^{1/2}V_{If}}{V_{II}} = 0.042/\text{min}\,. \end{aligned}\right\} \tag{4}$$

In addition, we know from our previous data [14] that

$$k_u = 0.101/\text{min} \tag{5}$$

and $t_{1/2} = 0.245\ C^{0.732}$ in serum, based on our previous data. Therefore,

$$k_i = \frac{\ln 2}{t_{1/2}} = 2.83\ C^{-0.732}\,. \tag{6}$$

Since the inactivation rate in the both compartments can be assumed to be the same because it is caused by blood,

$$k_{Ii} = 2.83\ C^{-0.732}, \quad k_{IIi} = 2.83\ C^{-0.732} \tag{7}$$

can be applied. It should be noted that the inactivation rate, in this case, is a function of the serum concentration. Substituting the parameter values obtained in eqs. (4–7) into eq. (3), one can obtain the following equation:

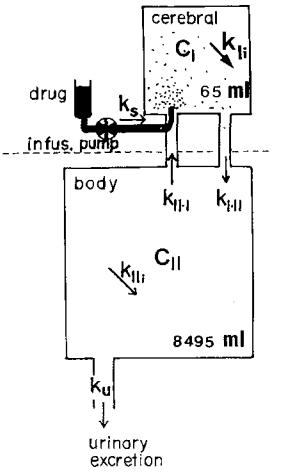

Fig. 1. Two-compartment model for the intra-arterial infusion of drugs for the treatment of brain tumor. C_I, drug concentration in the cerebral blood of a hemisphere which has a distribution volume of 65 ml; C_{II}, drug concentration in the systemic blood circulation outside of the cerebral blood circulation, which has an apparent volume of drug distribution of 8,495 ml. $k_{II\text{-}I}$ and $k_{I\text{-}II}$ are the transfer rate constants from the compartment II-I and I-II, respectively. k_u, urinary excretion rate constant; k_{Ii} and k_{IIi}, inactivation rate constants in the compartment I and II, where $t_{1/2}$ is near or less than 3s; k_s, infusion rate constant

$$\left.\begin{aligned} \frac{dC_I}{dt} &= -(5.54 + 2.83\ C_I^{-0.732}) \cdot C_I + 5.54\ C_{II} + \frac{k_s}{65}\,; \\ \frac{dC_{II}}{dt} &= 0.042\ C_I - (0.143 + 2.83\ C_{II}^{-0.732}) \cdot C_{II}\,. \end{aligned}\right\} \tag{8}$$

From in vitro data obtained in this study for glioblastoma, a minimum inhibitory concentration with respect to C_I can be found between 0.003 and 0.128 (μg/ml) and the maximum amount of drug to be infused is between 0.25 and 1.5 mg in total according to arbitrary toxicity data. Within these ranges, C_{II} is found to be almost close to zero from the simulation data. For example, $C_{II} = 10^{-14}$ μg/ml is obtained when $C_I = 0.1$ μg/ml and both are in the steady state. Hence eq. (8) can be written in the form;

$$\frac{dC_I}{dt} = -(5.54 + 2.83\ C_I^{-0.732}) \cdot C_I + \frac{k_s}{65}. \tag{9}$$

In the steady state $\dfrac{dC(t)}{dt}$ will be zero, thus

$$k_s = (5.54 + 2.83\ C_{Id}^{-0.732}) \cdot C_{Id}\, 65 \tag{10}$$

where C_{Id} denotes the desired concentration in the compartment I.

After simulation for the given C_I concentrations we obtained the results shown in Table 1.

The simulated results are described in the following section, in which each process of attaining the desired drug levels (transient state to steady state, $C(0) = 0$ to $C_{d(t)}$), is negligibly short compared with the time required for the infusion in these dose regimens. Thus, all transient processes, either at the initial or at the terminal stages, appear very similar despite the different dosing regimen.

Delay Compartment. Since the model described above is based on the assumption that NCS will be mixed to homogeneity almost

Table 1. Pharmacokinetic two-compartment simulation model for patients with brain tumor. Infusion velocity via internal carotid artery and designed intracerebral concentrations of NCS

Cerebral arterial concentration of the drug to attain (μg/ml) C_{1d}	Infusion rate constant *ks* (μg/min)	Amount of drug (mg)			
		0.25	0.5	1.0	1.5
		Infusion time required (min)			
0.0001	15.6	16.0	32.1	64.1	96.2
0.0002	18.8	13.3	26.6	53.2	79.8
0.0005	24.2	10.3	20.7	41.3	62.0
0.001	29.2	8.6	17.1	34.2	51.4
0.002	35.5	7.0	14.1	28.2	42.3
0.004	43.3	5.8	11.5	23.1	34.6
0.008	53.3	4.7	9.4	18.8	28.1
0.016	66.5	3.8	7.5	15.0	22.6
0.032	84.7	3.0	5.9	11.8	17.7
0.064	111.1	2.3	4.5	9.0	13.5
0.128	152.1	1.6	3.3	6.6	9.0

Data based on the human model of body weight of 60 kg. For calculation, see text

instantaneously in compartment II, this assumption needs to be corrected. For this reason a delay compartment was added to the model described. We found, however, virtually no difference in the simulated results even with an added compartment (see *Appendix*).

Results and Discussion

The present compartment model with NCS is based on the advantages NCS has over many other anticancer agents. It is cell cycle-specific [2, 8, 22], possesses a very short $t_{1/2}$ (this report; [15, 16]), is taken up at much higher rate into the cytosol and nucleus of the tumor or transformed cells than of normal cells [10, 20, 21], exhibits very high potency against glioblastoma cells (Fig. 2), and no crossing of the blood-brain barrier in the normal cerebral compartment (no CNS toxicity) [13]. In vitro, the minimum susceptibility of glioblastoma cells to NCS was found to be below 0.005 μg/ml, whereas normal glia cells (brain cells) were not affected at 0.3 μg/ml, as shown in Fig. 2. This result indicates that NCS is extremely effective against the glioblastoma cells, but is rather ineffective against non-dividing glia cells. Similar results were reported for lymphoblastoid cells and lymphocytes [10]. This result alone provides a rational basis of the selectivity of NCS to glioblastoma cells.

The inactivation half-times of NCS at various concentrations in CSF and in serum are shown in Fig. 3. The results revealed that the log of inactivation half-time is linearly correlated with the log of NCS concentrations. Therefore, the inactivation rate con-

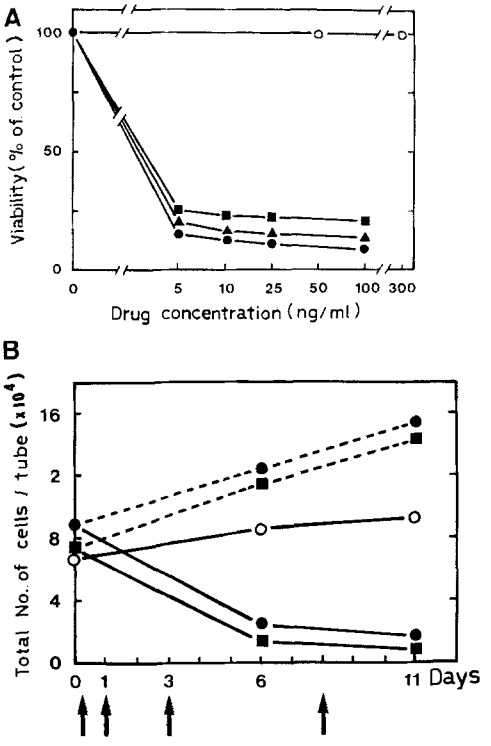

Fig. 2A and B. Effect of NCS on the glioblastoma and glia cells in culture. **A** Viabilities 12 days after cell culture in the presence of various concentrations and absence of NCS are shown. Drug treatments were carried out 4 h after cell plating and on days 1, 3, and 8 at the doses indicated. Each *point* represents an average of three assays: ■, ▲, ●, cell strains S-96, S-122, and S-155, respectively; ○, normal glia cells. **B** Effect of NCS on cell growth. *Arrows* indicate the time of addition of NCS. Final concentration was 0.005 μg/ml each. *Dashed lines* indicate glioblastoma cells, S-115 and S-96, without the drug. *Solid lines* show cells with drug treatment. In case of normal glia (brain) cells (○) there was no difference in the cell growth with or without drug treatment

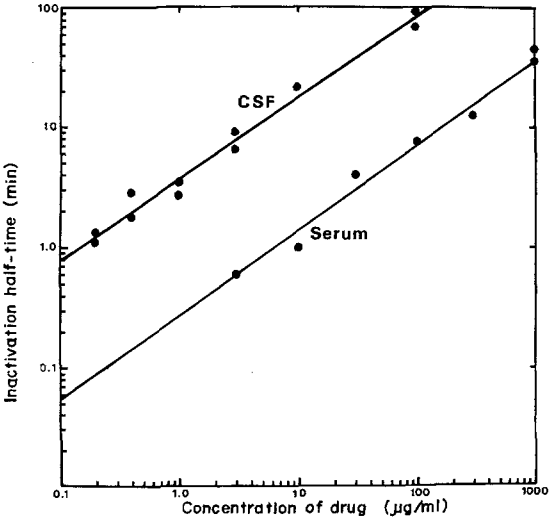

Fig. 3. Inactivation half-life of NCS in CSF and serum. The drug was diluted with CSF or serum to give the concentrations indicated, and each of the sample was incubated in darkness at 37° C followed by antibiotic assay [16]. The time required for 50% of the original activity ($t_{1/2}$) is plotted against each drug concentration

stant, k_i, varies in dependence on the drug concentration. From the data in Fig. 3 the relationship between the drug concentration and k_i was obtained by analysis of least squares, shown in eq. (6) for serum. The inactivation rate constant is about 16 times higher in serum than in CSF. This indicates that the drug is inactivated much more slowly in CSF. In parallel to this fact, the drug concentrations in the regional arteries (capillaries) of the tumors are maintained adequately high by the programmed continuous infusion into compartment I. Transcapillary leak to the lymphatic system is unique to NCS and has been established [16]. Thus the drug will leak effectively to CSF because of the deteriorated blood-brain barrier, and will remain active for longer than in the circulating blood.

The results of simulation for the two-compartment model are shown in Fig. 4 and Table 1. In the above-described two-compartment model, when the drug level in compartment I (C_I) is maintained at 0.1

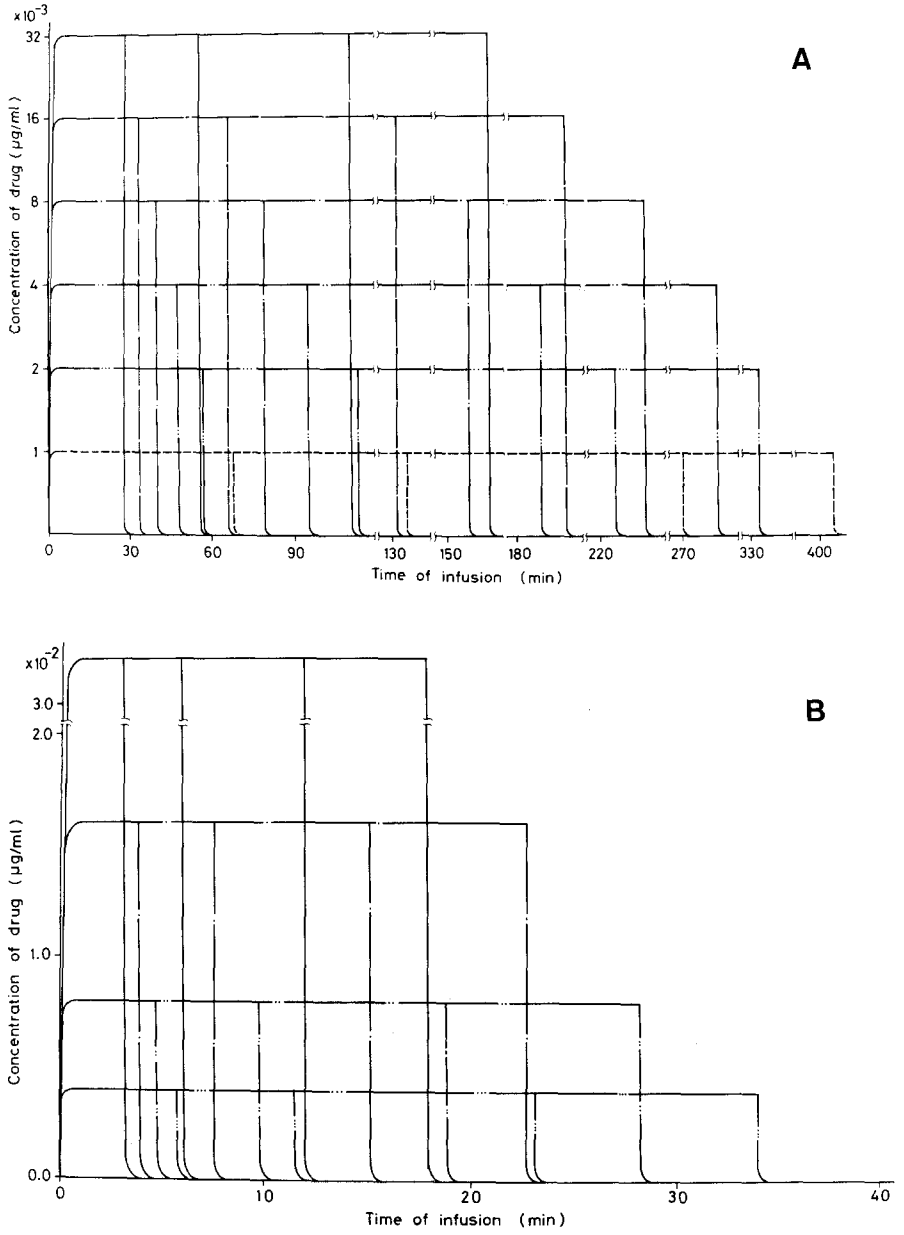

Fig. 4A and B. Pharmacokinetic simulation of infusion via internal carotid artery. The designed drug concentrations in the cerebral circulation are shown in horizontal levels. The completion of the infusion of each regimen, 0.25, 0.5, 1.0, and 1.5 mg of the drug, is shown by a vertical division from the horizontal levels. A rapid fall in drug concentration in the circulation upon the termination of each regimen is seen. **B** Similar to **A** but at the higher drug levels. Note that much shorter time is required for a given amount of drug to attain higher drug levels

μg/ml, that in compartment II (rest of the body) was found to be 10^{-14} μg/ml in the steady state. Although in compartment I the cerebral vascular space has the concentration C_I, we assumed here that the drug concentration in CSF, which is in contact with tumor cells, will become comparable to C_I. The reason for this assumption is that we know NCS can leak out of the capillaries and reach the interstitial space and, if the inactivation is slow, can remain there active (accumulation) without being recovered by the blood circulation though it is gradually recovered via the lymphatic system and then mixed with the circulating blood after the thoracic duct, then passing to the subclavian vein [16]. The NCS concentration in the lymph is known to become higher than that of the blood [16]. A similar mechanism was also observed in the renal clearance and accumulation in the urinary bladder via the urine, in which the $t_{1/2}$ is very long, followed by recirculation to the blood (vesicorenal recirculation) [14]. We anticipate, therefore, that the NCS concentration in CSF will be as high as C_I, since well-developed vascularities around the tumor tissues will facilitate such leak through the capillaries to the interstitial space (CSF). Thus, the concentration in CSF will be as high as that of C_I, or could be even higher than C_I.

As added in the *Appendix* a delay compartment having a delay time of 12 s was devised. The results of simulation showed that such a compartment was insignificant. This may be attributed to the fact that the $t_{1/2}$ for NCS in blood is extremely short (in C_{II}) at concentrations below 0.01 μg/ml (by extrapolation; $t_{1/2}$, 0.6 s) compared with the delay time of about 12 s. The $t_{1/2}$ in CSF at 0.01 μg/ml will be in the order of 10 s. In addition to the difference in k_i values, dilution from C_I (65 ml) to C_{II} (8,495 ml) results in a 130-fold decrease in the concentration.

This fact indicates that there will be no systemic side-effects of NCS if the infusion is carried out very slowly with a very small amount of the drug (0.25–1 mg). This is in a pronounced contrast to the regimen simulated for the complete remission of leukemia, in which 2 mg NCS was found to be infused *iv.* within a period of 4–10 min *iv.*, for instance, to attain a desired plasma concentration of 0.005–0.01 μg/ml in man.

The preliminary clinical results based on the present dose regimen of the two-compartment model showed that ten of 12 patients with brain tumor responded well to the NCS treatment [17]. In this clinical trial very little systemic or bone marrow toxicity was observed.

It is envisaged, however, that most of the chemotherapeutic agents used routinely, which possess much longer $t_{1/2}$ values than NCS or methotrexate, will result in an equilibrium between the two compartments. Therefore, it will not be an easy task to manipulate differential drug levels in the two separate compartments. In addition, other chemotherapeutic anticancer agents used for brain tumor such as CCNU, ACNU, and other alkylating agents, which are known to cross the blood-brain barrier, are also cytotoxic against non-dividing glia or nerve cells, and they are more or less independent of cell cycle, so that they may be accompanied by undesirable side-effects. Therefore, selective cytotoxicity will be very difficult to accomplish in the treatment of brain tumors with conventional drugs with longer half-lives, even though the pharmacokinetics are considered.

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Appendix: On a Delay Compartment Model

Since both $\frac{dC_I}{dt}$ and $\frac{dC_{II}}{dt}$ will be affected by the mutual delay terms, each delay time, τ_I and τ_{II}, may be incorporated in the eq. (8), which will yield the following equations:

$$\left.\begin{aligned}\frac{dC_{I(t)}}{dt} &= -[5.54 + 2.83\, C_{I(t)}^{-0.732}] \cdot C_{I(t)} \\ &\quad + 5.54\, C_{II(t-\tau_{II})} + \frac{k_s}{65}; \\ \frac{dC_{II(t)}}{dt} &= -[0.143 + 2.83\, C_{II(t)}^{-0.732}] \cdot C_{II(t)} \\ &\quad + 0.042\, C_{I(t-\tau_I)}\,.\end{aligned}\right\} \quad (11)$$

where τ_I and τ_{II} indicate delay time necessary for the equilibration of the drug concentration in the compartment I and II. Other parameters are similar to those described earlier in the text.

In numerical simulations of eq. [11], using the maximal expected concentration in C_I as 0.128 μg/ml and the delay times of τ_I and τ_{II} as 0.2 min, we obtained almost identical data with or without delay time as far as C_I is concerned (Fig. 5A). This can be expected because C_{II} (1.7×10^{-10} μg/ml) is far lower than C_I (an order of 10^{-1} μg/ml) to affect in C_I. A result obtained for C_{II}, however, did show a difference due to the delay time, as shown in Fig. 5B, which shows that C_{II} with a delay time requires a longer time to reach a steady state, depending upon the delay time. Therefore, as far as our system is concerned with NCS, the delay compartment model has very little significant meaning with regard to C_I.

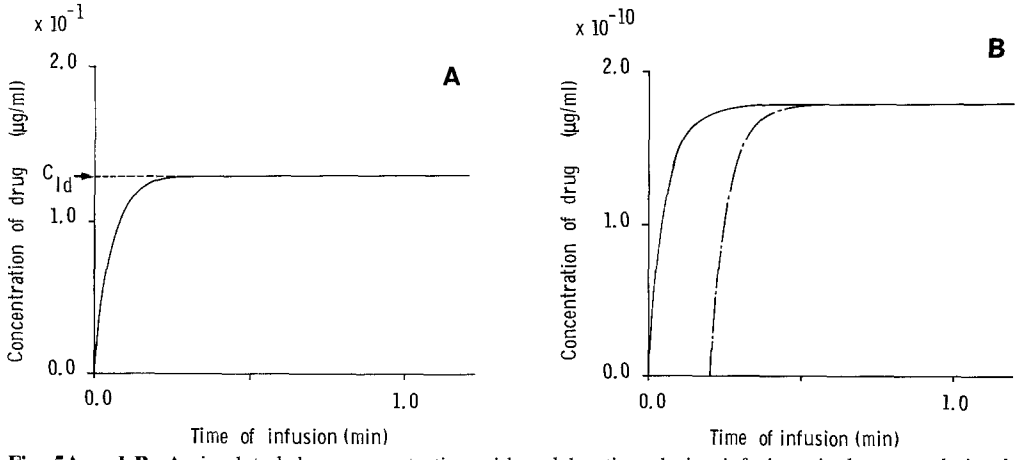

Fig. 5A and B. A simulated drug concentration with a delay time during infusion. **A** shows a relation between the concentration in compartment I (C_I) and time of infusion. In this case the desired drug level (C_{Id}) is 0.128 μg/ml in C_I. No difference was found between simulations with and without the delay time. **B** shows the similar relation in C_{II}. The drug concentration of the compartment II (C_{II}) with a delay time (*broken line*) reached exactly the same level as was attained without delay time (*solid line*) after an assumed delay time (0.2 min). All other parameters are described in the *Appendix* and main text