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Quantitative evaluation of targeted drug delivery systems

P.K. Gupta [1] and C.T. Hung [2]

[1] *College of Pharmacy, University of Kentucky, Lexington, KY 40536 (U.S.A.)*
and [2] *Zenith Technology Corporation Limited, Dunedin (New Zealand)*

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Summary

The methods of evaluating targeted drug delivery systems have been reviewed. It has been observed that in most instances the parameters used for the evaluation do not necessarily provide true quantitative differences between the selectivity of test and conventional delivery systems. It has been shown that the inadequate collection of data may lead to misinterpretation of the efficacy of drug delivery systems. Some mathematical relationships have been suggested and their usefulness substantiated with the aid of data describing the disposition of adriamycin administered to rats as a solution and via liposomes.

Introduction

Targeted drug delivery research is an area which concentrates on the development and evaluation of systems with precise characteristics. The characteristics sought may be selective or regional drug delivery, controlled drug delivery, or the combination of these characteristics (Florence and Halbert, 1985; Friend and Pangburn, 1987; Gardner, 1985; Gregoriadis, 1977; Gupta and Hung, 1989a; Poste and Kirsh, 1983; Poznansky and Juliano, 1984; Ranney, 1986; Sezaki and Hashida, 1984). Before any such delivery system can be made available for routine use, it is important that the evaluation procedures are critically established and the advantage over a conventional dosage form, if any, clearly documented.

A great deal of effort has been made towards the development and preclinical evaluation of microspheres, drug-conjugates, liposomes and similar systems, specially for the targeted delivery of cancer chemotherapeutic agents (Davis et al., 1984; Goldberg, 1983; Juliano, 1980; Tyle, 1988). The literature available on these systems often claim their higher efficacy in drug delivery compared to the delivery of an equivalent dose of drug as a solution. However, a critical screening of these reports indicates that very often the conclusion(s) regarding the superior efficacy of drug delivery systems have been based on very little data. In most instances, the efficacy of a test drug delivery system over conventional administration of drug has been determined by simply comparing the drug concentrations in selected tissues of an animal model at a limited number of time points after dosing. If radioactive carrier or drug is used then tissue radioactivity level, rather than drug concentration, is the parameter of choice. The results

Correspondence: P.K. Gupta, College of Pharmacy, University of Kentucky, Rose Street, Lexington, KY 40536-0082, U.S.A.

of such studies have often been expressed as (Bosworth and Hunt, 1982; Couvreur et al., 1980; DeLoach and Barton, 1981; Fujimoto et al., 1983, 1985; Juliano and Stamp, 1978; Kante et al., 1980; Kiwada et al., 1986; Kreuter and Hartman, 1983; Singhal and Gupta, 1986; Sugibayashi et al., 1977, 1979; Takada et al., 1984; Zimmermann et al., 1978):

Drug targeting index or Drug localization index
= (drug concentration or % radioactivity in i^{th} tissue at time t, after the administration of test drug delivery system) /(drug concentration or % radioactivity in i^{th} tissue at time t, after the administration of drug as a solution) (1)

Similar relationships have also been used to evaluate the effect of inclusion of magnetite, or altered surface characteristics of micro-carrier system, on their selective distribution in the body (Akasaka et al., 1988; Ibrahim et al., 1983; Illum and Davis, 1983, 1984, 1987; Morimoto et al., 1980, 1981; Mosbach and Schroder, 1979; Ovadia et al., 1983; Senyei et al., 1981; Widder et al., 1978). If the value of the ratio estimated using Eqn. 1 is greater than unity, even at one or two time points after dosing, the delivery system has usually been claimed to be specific and selective.

The use of Eqn. 1 with only one tissue, e.g. liver or tumour tissue, may not provide any indication about the reduction in drug toxicity to other tissues, which is often expected as a key advantage of targeted drug delivery systems. Hence some workers have determined the targeting or selectivity index by employing Eqn. 1 to the data obtained from target as well as non-target tissue(s) (Gipps et al., 1986; Grislain et al., 1983; Illum et al., 1984; Yoshioka et al., 1981), i.e.

Drug targeting index or Selectivity index
= (drug concentration or % radioactivity in target tissue at time t) /(drug concentration or % radioactivity in non-target tissue at time t) (2)

In fact, the literature available on the evaluation of targeted drug delivery systems indicates that few investigators have made an attempt to provide a detailed picture of the time course of drug, in all major tissues of the body, following its administration via the test and conventional delivery systems (Gallo et al., 1989; Gupta and Hung, 1989b; Gupta et al., 1986; Li et al., 1984; Papahadjopoulos and Gabizon, 1987; Rahman et al., 1984, 1986; Rosa and Clementi, 1983). This report highlights the implications of inadequate evaluation of targeted drug delivery systems. An example is presented to demonstrate the possibility of misinterpretation of the efficacy of targeted drug delivery systems due to the lack of sufficient data. Some simple mathematical relationships have been suggested which may be useful in gaining a better appreciation of the in vivo performance of targeted drug delivery systems. The application of these relationships has been briefly discussed with the aid of multiple tissue concentration-time data obtained following the administration of adriamycin as a solution and via cardiolipin liposomes to the rats.

Pharmacokinetic considerations

The most commonly accepted way to compare the efficacy of regional drug delivery, e.g. intra-arterial (i.a.) against intravenous (i.v.) administration of drug, is to obtain a quotient (Chen and Gross, 1980; Collins, 1984; Daemen et al., 1988a, 1988b; Hunt et al., 1986, 1988; Levin, 1986; McVie, 1984; Stephens, 1983; Weiss, 1985)

$$\text{Therapeutic benefit} = \frac{(C_{ss})_{i.a.}}{(C_{ss})_{i.v.}} \quad (3)$$

where C_{ss} represents drug concentration in a given tissue at steady-state. This quotient can be readily obtained following multiple dosing of a drug, with known pharmacokinetic behaviour, as a solution. However, the application of this relationship in the evaluation of effectiveness of a test targeted drug delivery system is difficult because in most situations only single dosing of the delivery device is attempted. Multiple dosing of a test targeted drug delivery system, especially if it is a colloidal carrier like microspheres, nanoparticles or drug-conjugate, is usually not attempted either due to

the lack of prior information about the toxic doses of the delivery device, or due to the possible alteration in the multiple tissue disposition of the second or subsequent doses of delivery device (Abra et al., 1980; Allen et al., 1984; Bosworth and Hunt, 1982; Ellens et al., 1983; Gregoriadis et al., 1977; Gupta and Hung, 1987; Hung et al., 1987; Sato et al., 1986; Souchami et al., 1981). A relationship frequently useful under these circumstances is (Eriksson and Tozer, 1986; Gallo et al., 1989)

$$r_e = \frac{(AUC_0^\infty)_{i,\text{ Test targeted drug delivery system}}}{(AUC_0^\infty)_{i,\text{ Conventional drug delivery system}}} \quad (4)$$

where the numerator refers to time-averaged exposure of any tissue i to drug administered via test targeted drug delivery system, the denominator refers to the time-averaged exposure of the same tissue following the administration of an equivalent dose of drug via a conventional drug delivery system, and r_e refers to the time-averaged relative drug exposure. Values of $r_e > 1$ indicate that the tissue 'i' is exposed to drug to a greater extent following the use of test targeted drug delivery system, and vice-versa. Although this relationship provides a good indication about the relative efficacy of two delivery systems in reference to one tissue, it does not provide any information regarding the efficacy of a given delivery device in terms of the target:non-target tissue distribution of drug. This information can be obtained using the following expression (Gallo et al., 1989)

$$t_e = \frac{(AUC_0^\infty)_{\text{Target-tissue}}}{(AUC_0^\infty)_{\text{Non-target tissue}}} \quad (5)$$

where t_e refers to the drug targeting efficiency of a delivery system against a given non-target tissue. For example when evaluating the targeting efficiency of a microparticulate drug delivery system in a tumour bearing animal, the target and non-target tissue can be best represented by the tumour tissue and the liver, respectively. Here values of $t_e > 1$ indicate greater selectivity of the delivery system for the target tissue, as compared to the non-target tissue against which this parameter is estimated. Implicitly, the higher the value of t_e, the greater is this selectivity. The ability of two delivery systems to modulate the target:non-target tissue distribution of a drug can therefore be readily compared as

$$r_{t_e} = \frac{(t_e)_{\text{Test targeted drug delivery system}}}{(t_e)_{\text{Conventional drug delivery system}}} \quad (6)$$

The key application of Eqns. 5 and 6 lies in situations where the targeting efficiency of a delivery system needs to be determined at two or more different dose levels, e.g. determination of dose-dependent kinetics of a delivery system, where the use of Eqn. 4 may not be acceptable. In addition, these relationships (Eqns. 5 and 6) may also be useful in obtaining some quantitative information regarding the efficacy of different doses of a drug administered via two different delivery systems.

In most instances, the drug concentration in tissue(s) is the parameter of choice to measure and compare the therapeutic or toxic levels of a drug, and this forms the basis of Eqns. 3 through 6. However, in true sense, targeted delivery systems are aimed at modulating the in vivo distribution of drug in such a manner that the maximum amount or the fraction of dose reaches the target cells (Hunt et al., 1986, 1988). In situations where the difference in the weight (or volume) of target and non-target tissues is marginal, the use of AUC_0^∞, and hence Eqns. 4 and 5, for the evaluation of targeted delivery system is adequate. However when the weight (or volume) of target and non-target tissue(s) is manyfold different, these relationships may not provide a true indication of the fraction of dose distributed to various tissues. A better appreciation regarding this issue can however be obtained by including a weight (or volume) term in these relationships. Eqn. 5 is then transformed to the following form

$$t_e^* = \frac{(AUC_0^\infty)_{\text{Target-tissue}} * (\text{weight or volume})_{\text{Target-tissue}}}{(AUC_0^\infty)_{\text{Non-target tissue}} * (\text{weight or volume})_{\text{Non-target tissue}}} \quad (7)$$

where t_e^* represent the weighted-average parameter similar to that described in Eqn. 5. Eqn. 7 can be further simplified to the form

$$t_e^* = \frac{(AUQ_0^\infty)_{\text{Target-tissue}}}{(AUQ_0^\infty)_{\text{Non-target tissue}}} \quad (8)$$

where AUQ_0^∞ is the area under the amount of drug vs time curve, which can be calculated in a manner analogous to AUC_0^∞. Here the amount of drug in a tissue, at any time t, is obtained as $Q = C^*V$, where C is the concentration of drug at time t and V is the volume of that tissue. In situations where more than one animal is used to obtain a data point, Q can be calculated as

$$Q = \frac{1}{n}(C_1V_1 + C_2V_2 + \ldots + C_nV_n) \quad (9)$$

where 1,2,... represent different animals and n is the total number of animals considered at that time. The use of Eqn. 8, instead of Eqn. 7, for the determination of t_e^* minimises the error introduced due to variation in the weight of different samples of a given tissue.

Whereas Eqn. 5 determines the drug targeting efficiency of a delivery system against a given non-target tissue, a composite or an overall drug targeting efficiency of a delivery system against n non-target tissues, T_e, can be calculated as

$$T_e = \frac{(AUC_0^\infty)_{\text{Target-tissue}}}{\sum_{i=1}^{n}(AUC_0^\infty)_i} \quad (10)$$

where the denominator refers to the sum total of drug exposure to all the tissues, including the target tissue. The corresponding weighted-average overall drug targeting efficiency (T_e^*) can therefore be determined as

$$T_e^* = \frac{(AUC_0^\infty)_{\text{Target-tissue}} * (\text{weight or volume})_{\text{Target-tissue}}}{\sum_{i=1}^{n}[(AUC_0^\infty)_i^* (\text{weight or volume})_i]} \quad (11)$$

or

$$T_e^* = \frac{(AUQ_0^\infty)_{\text{Target-tissue}}}{\sum_{i=1}^{n}(AUQ_0^\infty)_i} \quad (12)$$

The overall drug targeting efficiency of a similar dose of two different drug delivery systems, or two different doses of a delivery system, can then be compared as shown in Eqn. 6.

Finally, the time-averaged distribution of a drug to a given tissue, following the administration of a delivery system, can be estimated according to the relationship (Gupta and Hung, 1989b).

Drug distributed to tissue j (%) =

$$\frac{(AUC_0^\infty)_j^* (\text{weight or volume})_j}{\sum_{i=1}^{n}[(AUC_0^\infty)_i^* (\text{weight or volume})_i]} \times 100 \quad (13)$$

where j refers to any tissue in which drug distribution is determined. This equation can also be expressed as

Drug distributed to tissue j (%) =

$$\frac{(AUQ_0^\infty)_j}{\sum_{i=1}^{n}(AUQ_0^\infty)_i} \times 100 \quad (14)$$

or

Drug distributed to target-tissue (%)

$$= (T_e^*) \times 100 \quad (15)$$

Implicitly the practical usefulness of Eqns. 13–15 is based on the assumption that all the tissues involved in the distribution, metabolism and elimination of drug and drug–carrier complex are monitored in the study. This therefore means that the larger the value of n, the more satisfactory is the determination of this parameter (see Eqns. 11–14). In addition, the moment analysis of data, e.g. determination of mean residence time of drug in a given tissue following its administration via different delivery systems (Veng-Pedersen and

Gillespie, 1984; Yamaoka et al., 1978), in conjunction with the use of these relationships may increase the reliability of the interpretation of data. However purely from the standpoint of comparison of two delivery systems, or two doses of a delivery system, the collection of data from major tissues, and the use of Eqns. 13–15, may allow their adequate evaluation.

Materials and Methods

Data collection

One set of data, describing the distribution of daunorubicin to the liver and lung of mice, following its i.v. administration (4 mg/kg) as a free solution and via cardiolipin liposomes (see Table 1), was obtained from Rahman et al. (1984).

Another set of data on the multiple-tissue disposition of adriamycin, following its administration as a solution and via cardiolipin liposomes was also abstracted from the literature (Rahman et al., 1986).

Analysis of data

All the drug concentration data were analysed using a non-compartmental method to obtain total area under concentration-time curves (AUC_0^∞) (Gibaldi and Perrier, 1982). The area from time zero to the first concentration–time point in the post-distribution phase (C_t) was estimated using the trapezoidal rule, and the area from C_t to time infinity was determined as a quotient C_t/k, where k is the terminal disposition rate constant in units of reciprocal hours. The value of k was obtained by fitting linear regression to the log concentration-time data in the terminal phase (Gibaldi and Perrier, 1982). The total area from time zero to infinity (AUC_0^∞) was then obtained by adding the two values.

Different relationships (Eqns. 4–15) were then employed to interpret the data (see Results and Discussion). For solving the Eqns. 7, 11 and 13, the tissue weights were obtained from the literature (Gerlowski and Jain, 1983; Harrison and Gibaldi, 1977; Igari et al., 1983; Sakiya et al., 1985) and a tissue density of 1 g/ml was considered.

Results and Discussion

Table 1 lists the concentration-time data for daunorubicin in the liver and lung of mice, following the i.v. administration of its equitoxic doses as a solution and via cardiolipin liposomes. If this data were instead collected only up to 1 h for the liver, or up to 8 h for the lung, especially at insufficient time points to determine AUC_0^∞, one would conclude that compared to the solution delivery of drug the liposomes are more specific in the delivery of daunorubicin to liver, and they also reduce its delivery to lung. However, the comparison of their AUC_0^∞ (see Table 1) leads to altogether different conclusions, i.e. liposomes offer no advantage in the targeted delivery of drug to liver. In addition, this delivery system does not reduce exposure of lung to the drug. This example clearly highlights the likelihood of misinterpretation of results in situations where limited data are collected. This is not meant to say that direct comparison of tissue drug concentrations is inappropriate. However, it does indicate that comparison of tissue drug concentrations, following the administration of two delivery systems, may not provide conclusive evidence regarding their comparative behaviour unless the drug concentrations

TABLE 1

Daunorubicin concentrations (μg/g) and AUC_0^∞ (μg·h/g) in liver and lung of mice, following the i.v. administration of 4 mg/kg drug as a solution and via cardiolipin liposomes [a]

Time (h)	Liver		Lung	
	Solution	Liposomes	Solution	Liposomes
0.0833	22.10	22.30	11.00	9.75
0.167	15.20	20.40	12.60	7.96
0.5	12.10	14.60	13.00	7.11
1.0	9.87	11.50	8.20	5.10
1.5	12.10	10.40	6.71	5.17
2.0	9.67	8.46	7.28	5.77
4.0	6.87	6.28	3.82	3.20
8.0	2.99	2.68	2.47	1.78
24	1.28	0.92	0.45	0.93
AUC_0^∞ (μg·h/g)	105.10	96.64	68.81	68.65

[a] Adapted in part from Rahman et al. (1984), with permission.

in two groups vary by a large factor (for example see Debbs et al., 1987; Montgomery et al., 1988).

Table 2 summarises the AUC_0^∞ for different tissues of rat following the i.v. administration of 6 mg/kg adriamycin as a solution and via the cardiolipin liposomes. The Table also lists the time-averaged relative drug exposure values, determined using Eqn. 4. The r_e values indicate that the use of liposomes increased the exposure of liver to the drug by a factor of 26 and the exposure of heart to drug decreased by a factor of 2. Table 3 compares the targeting efficiency of liposomes and solution delivery system, as determined using Eqns. 5 and 7. The t_e values < 1 for the solution delivery system indicate that it exhibited little selectivity in terms of drug distribution to the liver. However, t_e values > 1 for the liposomal delivery system indicate that it selectively distributes drug to the liver. A t_e value of 52 against heart suggests that liposomes exhibit considerable discrimination between the liver and the cardiac tissue. In other words, this means that liposomes possess little selectivity towards heart as opposed to the liver. A t_e value of 1.58 against the spleen indicates that liposomes could not efficiently discriminate this tissue from the liver. The comparison between the t_e and the t_e^* values, for both delivery systems, indicates that except for the intestine, the t_e^* values are in general 5–20 times higher than the corresponding t_e values. This in turn reflects the difference in weight of the target (liver) and non-target tissues. Intestine, on the other hand, being a tissue with weight closely resembling that of the liver displayed little difference in t_e and t_e^* values. Interestingly, the t_e values for liposomes indicate that these particles were targeted to the spleen to the same extent as the liver ($t_e = 1.58$), and their delivery was considerably reduced to the intestine ($t_e = 28.12$). However, the t_e^* values for the same tissues indicate that the liposomes were in fact delivered to the intestine to a greater extent than the spleen (see Table 3). Hence the inclusion of weight terms in the determination of targeting efficiency may provide information which is often missed in routine analysis.

The data in Table 4 indicate that the liposomes exhibited 5 times higher overall drug targeting efficiency (T_e) than the solution delivery of drug. But if the weight of the tissues is taken into account, the T_e^* values suggest that liposomes were 3 times more efficient than the solution delivery system. This apparent decrease in the efficiency of liposomes, compared to that of the solution delivery of drug, can be explained in view

TABLE 2

Total area under adriamycin concentration – time curves (AUC_0^∞) in various tissues of rat, following the i.v. administration of 6 mg/kg drug as a solution and via the cardiolipin liposomes [a]

Tissue	AUC_0^∞ (μg·h/ml)		r_e
	Solution	Liposomes	
Heart	298.6	146.9	0.49
Liver	294.2	7652.6	26.01
Spleen	813.7	4826.2	5.93
Lung	318.2	488.7	1.54
Kidney	369.7	368.6	0.98
Intestine	425.9	272.1	0.64

[a] Adapted in part from Rahman et al. (1986), with permission.

TABLE 3

Drug targeting efficiency (t_e) and weighted-average drug targeting efficiency (t_e^*) of solution and liposomes in the delivery of 6 mg/kg adriamycin to the liver of rats [a]

Tissue	t_e		t_e^*	
	Solution	Liposomes	Solution	Liposomes
Heart	0.96	52.09	10.10	533.96
Liver	1.00	1.00	1.00	1.00
Spleen	0.36	1.58	7.41	32.50
Lung	0.92	15.66	7.84	132.83
Kidney	0.76	20.76	4.08	106.40
Intestine	0.69	28.12	0.57	23.22

[a] Determined using Eqns. 5 and 7, respectively.

TABLE 4

The overall drug targeting efficiency (T_e) and the weighted-average drug targeting efficiency (T_e^*) of solution and liposomes in the delivery of 6 mg/kg adriamycin to the liver of rats [a]

T_e		T_e^*	
Solution	Liposomes	Solution	Liposomes
0.117	0.556	0.298	0.915

[a] Determined using Eqns. 10 and 11, respectively.

TABLE 5

Percentage drug distributed to various tissues of rat following the i.v. administration of 6 mg/kg adriamycin as a solution and via liposomes [a]

Tissue	Solution	Liposomes	r_d [b]
Heart	2.9	0.2	0.07
Liver	29.8	91.5	3.07
Spleen	4.0	2.8	0.70
Lung	3.8	0.7	0.18
Kidney	7.3	0.9	0.12
Intestine	52.2	3.9	0.07

[a] Determined using Eqn. 13.

[b] Refers to relative distribution of drug, determined as a quotient, i.e., $r_d = (\%\ \text{drug distributed})_{\text{Liposomes}}/(\%\ \text{drug distributed})_{\text{Solution}}$.

of the fact that despite its relatively large weight, intestine was exposed to the liposomal drug to almost the same extent as kidney or heart (see Table 2). Hence the intestine must have received a comparatively greater fraction of the drug dose. This therefore decreased the weighted average efficiency of the delivery system.

Table 5 compares the time-averaged drug distribution to different tissues, following the i.v. administration of adriamycin as a solution and via the liposomes. In case of solution delivery, the intestine received ~ 2 times more drug than the liver. The use of liposomes increased the percentage of drug distributed to the liver by a factor of 3. The drug distribution to other tissues, including spleen, was reduced by 30–90%. The use of other relationships did not reveal the same information for either solution or the liposomal delivery of drug.

In conclusion, this report discusses various alternatives in which targeted drug delivery systems may be compared. It is believed that the use of some simple mathematical relationships may allow better understanding of the in vivo performance of drug delivery systems. The proposed relationships can be applied to evaluate the efficacy of comparable doses of drug delivered via two systems, as well as to evaluate the efficacy of a system in the delivery of different doses of a drug. It is realized that in most situations drug concentration rather than the amount of drug in a tissue is responsible for the biological effect. However, with the rapidly growing interest in the area of selective delivery of highly toxic molecules, e.g. use of ricin in cancer chemotherapy (Carriere et al., 1985; Embleton, 1986; Sezaki and Hashida, 1984), the precise quantitative evaluation of the efficacy of delivery system(s) is undoubtedly important. Under such circumstances, the application of relationships discussed in this report may be more reliable than simply comparing the drug concentrations in various tissues.

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