Proc. Indian natn. Sci. Acad., **B60**, No. 4, pp. 345-356 (1994)

Receptor-Mediated Drug Delivery to Macrophages

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Toxic side effects resulting from the administration of therapeutic agents often arise due to the fact that the non-target cells in the body are also exposed to the cytotoxic effects of the drugs. Targeting of drugs selectively to the cells where pharmacological action is desired is expected to increase their therapeutic efficacy and decrease the toxic side effects. A promising approach to site-specific drug delivery consists of attaching the therapeutic agent to a determinant unit recognized only by the cells where pharmacological action is desired. For this purpose, we have been exploiting the exquisite cell type specificity and high efficiency of endocytosis of macromolecules mediated by specific receptors present on the surface of some mammalian cell types. As macrophages are affected in a large number of viral, bacterial, fungal, protozoal, metabolic and neoplastic diseases, we have demonstrated the feasibility of this approach for delivering drugs selectively to these cells. Our approach consists of chemical coupling of an appropriate drug to a carrier molecule, *e.g.*, maleylated albumin which is recognized by specific receptors (called scavenger receptors) present exclusively on cells of macrophage lineage. We have shown that such drug conjugates bind with high affinity to the scavenger receptors on macrophage surface leading to rapid internalization and subsequent degradation of the ligand in lysosomes releasing a pharmacologically active form of the drug. So far we have tested the efficacy of the approach in cell culture and animal models of macrophage-associated disorders of protozoal (leishmaniasis), bacterial (tuberculosis) and neoplastic etiology. In all three instances examined, the conjugated drug was nearly 100-fold as effective as the free drug. The results indicate that the receptor-mediated modality of delivering drug to macrophages could contribute to greater therapeutic efficacy and minimization of toxic side effects in the management of intracellular infections as well as neoplastic diseases. Maleylated albumin-mediated delivery of various agents also provides a generalized tool for manipulating the metabolic activity of macrophages for a variety of purposes.

Key Words: Endocytosis; Scavenger receptor; Drug targeting; Macrophage-associated disease

Introduction

Basically, two approaches are in vogue for site-specific drug delivery:

(i) *The prodrug approach*: In this chemical derivatives of the drugs are prepared that have more favourable kinetic features and/or are specifically activated at the site of action.

(ii) *The carrier approach*: In this the drug is covalently coupled to a soluble macromolecule or included in a particulate type of drug carrier. The fate of the drug in the body is dictated by the chosen carrier. The drug concentration in the target tissue is a result of the relative rates of cellular uptake of the drug conjugate, liberation of the drug, and efflux rate of free drug from the target tissue. An efficient drug carrier must have the properties as detailed in table 1.

Depending on the intended use both particulate and soluble types of carriers are being developed. Particulate carriers include synthetic polymeric nanoparticles, protein microspheres, viral or cellular matrices,

Table 1 *Properties of an ideal drug carrier*

—It must be able to cross anatomic barriers.
—It must be recognised specifically by the target cells.
—The linkage between the drug and the directing unit should be stable in the plasma and extracellular spaces.
—After recognition and internalisation of the drug carrier systems, active pharmacological drug unit must be released inside the target cells.
—The carrier should be nontoxic and nonimmunogenic in the host.
—The conjugate should be manufacturable under sterile, apyrogenic condition.

multiphase microemulsions, and liposomes. The particulate carriers permit loading of relatively large amounts (around 500 drug molecules per particle) of the drug without covalent linkage with the carrier and can carry both water-soluble and lipid-soluble drugs. Such carriers, however, can not pass through the endothelial lining and their extravasation is generally poor. Also, these particles are efficiently captured by the mononuclear phagocyte system either in the parent or in the opsonized form which markedly restrict their contact time with the target cells. Furthermore, if the phagocytosed materials are not degraded rapidly enough, blockage of the mononuclear phagocyte system function may result in chronic toxicity, especially with repeated administration. Use of such carriers, therefore, appears to be limited to specific applications to localized areas of the body. Soluble drug carriers usually have relatively low loading capacity (less than 50 drug molecules per carrier) but suitable for both vascular as well as extravascular applications as they can easily leave circulation and can reach target cells not in direct contact with blood.

Several monographs and reviews are available on the problems and prospects of various approaches for selective drug delivery (Poznansky & Juliano 1984, Juliano 1991, Tomlinson & David 1986 and Langer 1990). In this article we shall discuss (i) the currently available strategies for cell type-specific targeting of soluble macromolecular carriers with special emphasis on targeting of drugs to macrophages (Basu 1990), and (ii) describe the scavenger receptor-mediated stratagem for selective intracellular delivery of a variety of pharmacologically active agents to macrophages for various purposes developed in our laboratory over the last several years.

Site-specific Targeting

Two distinct types of recognition elements on the surface of the target cells appear to be particularly promising in designing carriers for selective drug delivery, *viz.*, (i) cell surface antigens capable of generating specific, non-cross-reactive antibodies, and (ii) cell surface receptors capable of efficient transport of the macromolecular ligands.

In theory, monoclonal antibody mediated drug delivery appears to be the most versatile and universally applicable approach for site specific drug delivery. The initial promise of this approach still remains to be fulfilled because of a number of disadvantages as outlined in table 2. However, it is expected that with the advances in cell biology and immunology it should be possible to overcome many of these limitations. For example, it should be possible to incorporate appropriate screening protocols to identify clones producing antibodies which are efficiently internalized as also uniquely specific for a particular target cell type. Humanized antibodies would mitigate the problem of immunogenicity. The early euphoria regarding this approach has been replaced with a quiet optimism and

considerable effort continues to be mounted to make this approach a successful one.

Meanwhile, the spectacular advances in membrane biology research over the last two decades have provided intimate knowledge about an important high efficiency process used by the mammalian cells for the transport of macromolecules, *viz.* receptor mediated endocytosis, which serves to selectively retrieve and assimilate various macromolecules from the extracellular environment with high efficiency for a variety of purposes. The high efficiency of the process of receptor-mediated endocytosis results from the existence of specific cell surface receptors which recognize and bind specific macromolecules to the cell surface with high affinity. Following binding to the receptors the ligands are internalized with remarkable efficiency through the operation of a vesicular transport system, the essential elements of which first became apparent from the studies of Goldstein, Brown and their colleagues on the uptake of low density lipoprotein (LDL) by cultured human fibroblasts (Goldstein et al. 1983, Basu 1984). Studies over the last decade with a large number of systems have shown that after internalization, the receptor-ligand complexes, in general, are routed through one or the other of the following four pathways with distinct consequences for the receptor and the ligand:

1. Receptors recycle back to the plasma membrane, ligands get delivered to lysosomes,
2. Both the receptors and the ligands recycle to the plasma membrane,
3. Both receptors and ligands are transported to lysosomes,
4. Receptors are degraded, ligands are transported across the cells.

However, factors such as cell type, distribution of receptors, and ligand concentration can significantly affect the intracellular routing of the receptor-ligand complexes (Shepherd 1989). Nevertheless, this process appears to have several characteristics ideally suited for site-specific drug delivery applications (table 3).

Detailed information about the nature of the ligand, relative distribution of the receptor on various cell types, and the intracellular pathways followed by the receptor ligand complexes are, therefore, essential prequisites for adaptation of

Table 2 *Antibody mediated targeting: Disadvantages*

—Cross-reactivity of antibodies with the non-target cells.
—Limited access of antibody preparation to the target cell type.
—Inefficient internalization of antibody-drug conjugates in general.
—Cell heterogeneity with respect to the determinant antigen to which the antibody is directed.
—Immunogenicity of the antibody and especially that of antibody-drug conjugate.
—Opsonization of injected antibodies and complex formation with circulatory antigens.

Table 3 *Receptor-mediated endocytosis: Characteristics important for site-specific drug delivery*

—High affinity of the receptor for the ligand permits effective sequestration of the ligand present at low concentrations in the extracellular space.
—Rapid recycling of the receptors results in relatively high intracellular concentrations.
—Expression of specific receptors exclusively on certain cell types permit design of cell type specific drug delivery system.
—Depending on the nature of receptor-ligand complex, specific intracellular localization of the ligand (e.g., to the lysosomes, nucleus, cytosol or transport across the cells) may be possible.

receptor-mediated endocytotic processes for selective drug delivery. Unfortunately, most of the receptor systems for which such information is available at present are distributed on many cell types or ubiquitously. Attempts have been made to exploit the quantitative differences in the content of such receptors for targeting of antineoplastic drugs to cancer cells. At present, only hepatocytes and the cells of macrophage lineage seem to possess efficient receptor systems restricted largely to these cell types and amenable to selective drug delivery (Fallon & Schwartz 1989, Gordon & Rabinowitz 1989).

Macrophage as Target Cells

Macrophages have a pivotal role in mounting multipronged defensive responses which serve to protect the host against a wide variety of invading microorganisms and developing neoplasms through triggering of humoral and cell-mediated immune responses as well as intracellular oxidative or hydrolytic activities (Lewis & McGee 1992). However, in a number of microbial and metabolic diseases these protective responses are overwhelmed or circumvented so that these protective cells become the focal point in a large number of disease states, some examples of which are cited in table 4.

For diseases of microbial etiology, the intracellular localization of the pathogens necessitate administration of relatively high dosages of cytotoxic drugs for effective killing of the pathogens thereby causing side reactions which are often unacceptably severe. Such undesirable side effects presumably arise from the interaction of drugs with healthy normal cells. Since, macrophages are the primary affected host cell types in a number of viral, bacterial, parasitic, neoplastic and metabolic diseases (Mogensen 1979, Kleinerman et al. 1983, Fidler 1984), the ability to deliver antiviral, antibacterial, antiparasitic and antineoplastic agents specifically to macrophages would be of tremendous importance in the management of such diseases.

Table 4 *Diseases affecting macrophages: Examples*

Etiology	Diseases
1. Protozoal	Leishmaniasis, Toxoplasmosis.
2. acterial	Tuberculosis, Leprosy, Salmonellosis, rucellosis, Listerosis.
3. Neoplastic	Monocytic leukemia, Histiocytosis,
4. Viral	Dengue, Measles, AIDS, Japanese encephalitis.
5. Metabolic	Gaucher's disease, Rheumatoid arthritis

Choice of Macrophage Specific Receptor System

A large variety of receptor systems with important roles in controlling growth, differentiation, endocytosis, secretion and cell activation have been reported to be expressed on macrophages (Lewis & McGee 1992). Among the receptor systems on the surface of the macrophages, the mannosyl/fucosyl receptors, (Pantow et al. 1993) the galactosyl particle receptors on Kupffer cells (Bijsterbosch & Van Berkel 1990) and the scavenger receptors (Goldstein et al. 1979, Brown et al. 1980) are the most well characterised. All three receptors are probably recycled many times resulting in efficient intracellular delivery of the ligand. However, mannose receptor system is known to be downregulated under various conditions such as malignant transformation or infection with *Leishmania donovani* thereby limiting its use as a generic carrier of drugs to macrophages (Pantow et al. 1993, Basu et al. 1991).

Macrophages from different organs of a variety of animals including man express high-affinity binding sites, generically referred to as the scavenger receptors, which recognize a number of structurally diverse

polyanionic macromolecules such as modified proteins (e.g., acetylated or oxidised LDL, maleylated serum albumin), certain polysaccharides (e.g., fucoidin, dextran sulphate), polynucleotides (e.g., polyinosinic and polyguanylic acids), certain phospholipids, (e.g. fucoidin, dextran sulphate), polynucleotide (e.g. polyinosinic and polyguanylic acids), certain phospholipids phosphatidylserine), polyvinyl sulphate and bacterial lipopolysaccharides (Goldstein et al. 1979, Brown et al. 1980, Nishikama et al. 1990, Zhang et al. 1993). Molecular cloning of scavenger receptors from bovine, murine and human sources and sequencing have shown that they comprise of several descrete molecular species with characteristic ligand binding properties (Krieger 1992). Binding of protein ligands to the scavenger receptors is followed by internalization and lysosomal degradation of the protein ligands (Goldstein et al. 1979, Brown et al. 1980). The scavenger receptor mediated endocytic pathway possesses the essential elements which can be exploited to design an efficient and selective drug delivery system for cells of macrophage lineage as shown in table 5. Scavenger receptor ligands recognised to date are polyanionic macromolecules although many polyanions are known which are not ligands (Goldstein et al. 1979, Brown et al. 1980). The multiplicity of possible ligands for this receptor system provides considerable flexibility for designing carriers for different purposes.

Selective Delivery of Drugs to Macrophages Exploiting Scavenger Receptor System

We have been exploiting the exquisite cell-type specificity and high efficiency of scavenger receptor-mediated endocytic process for selective delivery of a variety of substances to cells of macrophage lineage to establish the feasibility of a common carrier useful for macrophage associated disorders in general which include protozoal, bacterial, viral, neoplastic and metabolic diseases. Our conceptual approach to the problem consists of chemical conjugation of the pharmacologically active agent appropriate for the disease in question with a ligand recognized by the scavenger receptors and determining the efficacy of the drug conjugate in comparison to the conventional modality of administration for the drug both *in vitro* and *in vivo* in appropriate cell culture or animal models. Our work so far has established the superior efficacy of various drugs conjugated with maleylated bovine serum albumin (MBSA) in model systems of a protozoal (leishmaniasis), a bacterial (tuberculosis), and a neoplastic (histiocytic malignancy) disease in which macrophages are involved (Mukhopadhyay et al. 1989, 1992, 1993 Chaudhuri et al. 1989, Mukhopadyay & Basu 1990, Majumdar & Basu 1991).

Leishmaniasis: A Protozoal Disease: Leishmaniasis, a group of protozoal diseases causes by various *Leishmania* species, affect 400,000 to 12 million people worldwide each year (Chance 1981). Moreover, the current resurgence of leishmaniasis in different parts of the world is a major public health concern. Visceral leishmaniasis, caused by *L. donovani* (called *Kala azar* in India) is fast assuming epidemic proportions in India and shows every symptoms of going beyond control. The life cycle of leishmania has a

Table 5 *Properties of scavenger receptor system*

—Macrophages and to a lesser extent endothelial cells express this receptor system.
—Binding sites are present on the cell surface.
—These receptors mediate rapid internalization and efficient degradation of the protein ligands.
—These receptors are not subject to down regulation and are recycled.
—Multiple rounds of receptor recycling might help in building up of high intracellular concentration of drugs.

cyclical development, i.e. an alternation of intracellular growth in a vertebrate host and extracellular growth in an insect. The extracellular flagellated form in the insect is known as promastigote while the intracellular nonmotile form in the macrophages is called amastigotes. The amastigate form resides and proliferates solely within macrophages which eventually rupture (Chang 1983). The liberated parasites infect fresh cells and the cycle is repeated. The entire reticulo-endothelial system becomes progressively affected and culminates in the clinical pathology and the symptoms associated with the disease. Thus, the amastigotes residing in macrophages are the target cells in the chemotherapy of leishmaniasis. Current drugs of choice for leishmaniasis, *viz.*, antimonials, amphotericin B and pentamidine, can produce severe side effects (Chang & Bray 1985).

We have shown that methotrexate (MTX) coupled with MBSA binds with high affinity to receptors on hamster peritoneal macrophages leading to rapid internalization and subsequent degradation of the ligand in the lysosomes. The drug conjugate was about 100 fold as effective as free MTX in eliminating the intracellular amastigotes of *L. donovani* and *L. mexicana amazonensis*. Data presented in table 6 show that the leishmanicidal activity of MBSA-MTX was primarily due to its content of MTX. MBSA alone had a small antileishmanial effect at high concentrations.

The leishmanicidal action of MBSA-MTX could be eliminated by simultaneous addition of an excess of MBSA to the medium reflecting that the uptake of MBSA-MTX occurred through a limited number of binding sites which recognize MBSA. Furthermore, the leishmanicidal activity of MBSA-MTX could be largely suppressed by the addition of chloroquine and monensin which are known to inhibit lysosomal functions indicating that the antileishmanial product was released from MBSA-MTX after lysosomal degradation of the drug conjugate. Finally, the amastigotes could be protected from the killing effect of MBSA-MTX in presence of folinic acid, the metabolite whose production was prevented by MTX, reflecting that the intracellular product generated by lysosomal degradation of MBSA-MTX acted like MTX. Our studies suggest that the superior antileishmanial effect of the drug conjugate in cultured hamster peritoneal macrophages in culture was due to enhanced uptake of the drug in the conjugated form through the scavenger receptor mediated uptake process which recognizes the MBSA moiety of the conjugate.

Studies were carried out to determine the efficacy of the drug conjugate in the treatment of cutaneous leishmaniasis using hamster foot-pad lesions as the experimental model system. In this study, the drug conjugate brought about more than 90% reduction in the size of the foot-pad lesion within 11 days after initiation of treatment. In contrast, same dosage of the free drug did not significantly affect the lesion size. (Figure 1). All the animals remained healthy and no

Table 6 *Inhibition of antileishmanial activity of MBSA-MTX by MBSA, lysosomotropic agents and folinic acid in* ***L. mexicana amazonensis*** *infected macrophages* (Mukhopadhyay and Basu 1990)

Additions	Amastigote survival (%)	
	Without MBSA-MTX	With MBSA-MTX (3 μg/ml)
None	100	14±5.5
MBSA		
100 μg/ml	81±3.2	64±3.4
200 μg/ml	82±2.9	79.±2.9
500 μg/ml	76±4.1	79±2.9
Chloroquine (3 μM)	68±6.3	59±5.8
Monensin (3 μM)	81±7.2	79±6.6
Folinic Acid (10 μg/ml)	99±2.5	100±3.2

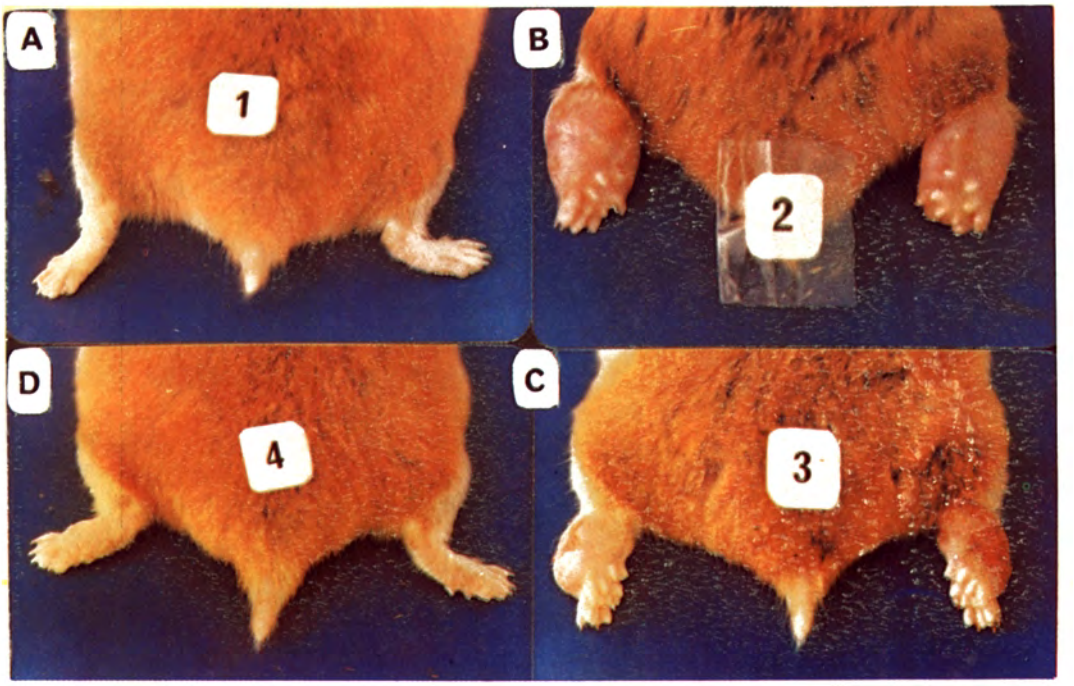

Figure 1 Regression of hamster footpad lesions after treatment with MBSA-MTX or MTX. The experiment was performed as described by Mukhopadhyay et al. (1989). Photographs of hind footpads of one representative animal from each group. A: Unifected; B: Infected, untreated; C: Infected, treated with free MTX (1 mg/kg body weght); D: Infected, treated with MBSA-MTX (1 mg/kg body weight MTX equivalent).

antibody response to the drug conjugate was elicited during the experimental period.

Concurrent with or subsequent to our work (Mukhopadhyay et al. 1989 Chaudhuri et al. 1989, Mukhopadhyay & Basu 1990) a few attempts have been made for selective killing of intracellular amastigotes of *Leishmania* using carriers such as native or altered low density lipoproteins, mannosylated neoglycoproteins, and yeast mannan (Hart & Lawrence 1990, Chakraborti et al. 1990, Cantos et al. 1993). In our opinion, exploitation of the scavenger receptor system offers several crucial advantages for selective delivery of antileishmanial drugs as alluded to earlier in this article.

Tuberculosis: A Bacterial Disease: Tuberculosis is a major public health concern, particularly in the developing countries (Sudre et al. 1992). Also the incidence of tuberculosis is increasing in United States in patients with AIDS, most likely due to reactivation of the latent infection with *Mycobacterium tuberculosis* (Bloom & Murray 1992). This bacteria owes its pathogenicity to its ability to survive and proliferate within mononuclear phagocytes of the infected host (Haque 1990). The success of current therapy for tuberculosis and other mycobacterial diseases, requires prolonged treatment with relatively high dosages of drugs in order to achieve effective intracellular concentrations in infected cells and often results in severe toxic side effects (Zierski & Bek 1980). Liposomal delivery systems used so far for antimycobacterial uses have not been very successful (Cynamon et al. 1989, Duzgunes et al. 1991). Therefore, an alternate approach for the selective delivery of antimycobacterial agents may be of greater help in controlling tuberculosis. Furthermore, in view of the fact that strains

of *M. tuberculosis* resistant to currently used antitubercular drugs are emerging, an efficient intracellular delivery system would considerably enhance the possibility of recruiting known but highly toxic antimycobacterials for therapy of tuberculosis.

With this notion, we have examined the feasibility of delivering an antitubercular drug, p-amino salicylic acid (PAS), to macrophages using MBSA as the drug carrier. This drug conjugate is recognised by the scavenger receptors present on the macrophages and causes selective elimination of the intracellular mycobacteria (Majumdar & Basu 1991). The data in table 7 show the antimycobacterial effect of different concentrations of PAS in free or conjugated form on the proliferation of *M. tuberculosis* in mouse macrophage cell lines. We have also shown that the drug conjugate at low concentrations was nearly 100 fold as effective as free PAS in eliminating *M. tuberculosis* H37Ra harbored by cultured mouse peritoneal macrophages. Thus, 50% reduction of the colony forming units was achieved by using 0.2 μg of the PAS in conjugated form, whereas the free form reduced only 0.5% of CFU (Majumdar & Basu 1991).

Table 7 *Effect of PAS-MBSA on the intracellular growth of M. tuberculosis $H_{37}Ra$ within J774A.1 and P388D1 cells*

Concentration (μg/ml)	J774A.1		P388D1	
	CFU/Well, 10^3		CFU/Well, 10^3	
	PAS	PAS-MBSA	PAS	PAS-MBSA
0	823	823	853	853
0.2	842	533	826	621
2	801	500	798	597
5	766	410	735	521
8	733	338	687	436
10	663	319	602	397
15	602	288	553	358

Macrophage monolayers were infected with *M. tuberculosis* (10 bacteria per macrophage), incubated at 37°C in a humidified incubator containing 5% CO_2. After 4 hr, the extracellular bacilli were removed by three washings with PBS, and incubated in RPMI medium containing 10% PCS for 24 hr. The indicated concentrations of PAS in free or conjugated form were added to the infected monolayers. After incubation for 4 hr at 37°C, the monolayers were washed three times and were reincubated for 48 hr in drug free medium. Cell lysates were processed for determination of colony forming units as described before (Majumdar & Basu 1991)

Preliminary studies in an animal model in which guinea pigs were infected with *M. tuberculosis* $H_{37}Rv$ show that I.V. administration of PAS-MBSA conjugate led to persistent and significantly higher level of PAS activity in the lung, liver and spleen than the drug activity obtained in the free PAS-treated group. PAS-MBSA treatment effectively reduced the bacterial load in the infected organs and significantly increased the survival rate of the infected animals. Our studies thus show that it is possible to increase the antimycobacterial efficacy of PAS through conjugation of the drug to MBSA. Further studies will be necessary to establish the value of our approach in reducing dosage regimens and toxic side effects of drugs used in the chemotherapy of tuberculosis and other mycobacterial diseases.

Histiocytosis: A Neoplastic Disorder: The macrophage neoplasia or histiocytic malignancies are aggressive disorders and often fatal if untreated (Cline 1980). These diseases arise spontaneously in mice and humans and have been induced in mice by oncogenic viruses. In humans, these diseases are heterogeneous and expressed in different stages of the mononuclear phagocyte differentiation, e.g. acute monocytic leukemia (monoblasts, promonocytes), chronic monocytic leukemia (promonocytes, mature macrophages), histiocytic medullary reticulosis (promonocytes, immature macrophages), Letterer-Siwe disease (monoblast, immature macrophages) and eosinophilic granuloma of bone (immature and mature macrophages).

Moreover, these diseases also affect the functions of phagocytic leukocytes to render the host more susceptible to opportunistic pathogens. Fortunately, histiocytic malignancies are rare and the primary approach to combat these diseases lies with chemotherapeutic measures. However, reports of the response to single therapeutic modalities in these diseases are fragmentary and no well documented scientific literature is available. Although promising results have been obtained with combination chemotherapy, the toxicity associated with the drugs remains the major limitation of such drug combinations. Thus, there is a need to develop more effective chemotherapeutic regimens against these diseases.

We have recently investigated the efficacy of scavenger receptor-mediated delivery of an effective but highly toxic anticancer drug daunomycin, in cell culture and animal models of macrophage-associated neoplasia (Mukhopadhyay et al. 1992, 1993). We have shown that transformed cells of nonmacrophage lineage, viz. L929, EL4, Bowes melanoma and CHO, do not take up and degrade the conjugate, indicating that these cells are scavenger receptor deficient. Conjugation of DNM with MBSA significantly increased the cytotoxic activity of the DNM for the scavenger receptor bearing cells. Thus, 0.1 μM of the DNM in the conjugated form killed about 50% of the receptor positive cells, whereas free DNM at the same concentration killed less-than 0.5% of these cells. Therefore, the drug conjugate was at least 100 fold as effective as the free drug in killing the receptor bearing cells. But the receptor deficient Bowes melanoma cells were not affected by the drug conjugate. Similar results were obtained when human histiocytic lymphoma cells in culture were exposed to a conjugate of doxorubicin (DXR) with MBSA.

MBSA-DNM conjugate suppressed the growth of the solid tumours induced by J774A.1 in BALB/C mice at much lower dosage of DNM relative to the free form of the drug (Mukhopadhyay et al. 1993). Table 8 shows the mean survival time of the tumour bearing mice. The data show that the control mice which received only PBS died within 25 days, administration of free DNM in dosages (10-40 μg) improved the survival time only to a maximum period of 40 days. In contrast, the tumour bearing mice which received 40 μg of DNM in the conjugated form survived throughout the experimental period of 230 days reflecting the superior anti-tumour effect of the conjugate.

The results described above indicate the utility of our approach to control the proliferation of neoplastic cells of macrophage lineage which bear the scavenger receptors. However, as macrophages are known to migrate to solid tumour sites, we are currently exploring whether our approach of MBSA-drug conjugates can be extended to other types of tumours as well where the drug loaded macrophages would act as local slow-release depots of drugs. It is also conceivable that macrophages loaded with drugs or activated through the delivery of appropriate

Table 8 *Survival of tumour bearing mice treated with drug conjugate*

Treatment	No. of days	Mean survival time (days)
P S	5	22±2
M SA	5	23±2
DNM (10 μg)	5	36±8
DNM (20 μg)	5	39±2
DNM (40 μg)	5	38±8
M SA-DNM (10 μg)	5	162±9
M SA-DNM (20 μg)	5	230±0
M SA-DNM (40 μg)	5	230±0

AL /C mice were transplanted intraperitoneally with 5×10^4 J774A.1 cells on day 0, and subsequently treated with the indicated dosage of the drugs either in free or conjugated form on day 2, 3, 4 and 5. Results are expressed as the mean survival time after the observed period of 230 days.

modulatory agents using our approach would be effective in arresting proliferation of circulating cancer cells as pertains in metastasis.

The approach of receptor-mediated drug delivery to cancer cells using various receptor systems for lipoproteins, growth factors, cytokines, etc. is an active area of investigation (Basu 1990). Interesting *in vitro* results have been obtained using LDL (Lundberg 1987), growth factors (Cawley et al. 1980) and cytokines (Ogata et al. 1988) as drug carriers. However, since these receptors are also found on many types of normal cells, it is likely that drugs delivered through such receptors would not be targeted exclusively to the tumour cells. Administration of acetylated LDL containing a lipophilic derivative of muramyl tripeptide, which is taken up through the scavenger receptors, has been shown to enhance the tumouricidal activity of macrophages *in vivo* (Shaw et al. 1988). However, lipoproteins, growth factors and cytokines are difficult to obtain in large quantities and to formulate into stable pharmacological preparations. Anti-receptor antibodies to growth hormones which could be prepared in adequate quantities at reasonable cost provides an additional resource which could prove useful (Hirota et al. 1989). MBSA as a drug carrier is attractive because of simplicity of preparation, longer shelf life and ease of sterilisation and formulation into apyrogenic preparations.

Conclusion

The concept of using receptor-mediated endocytotic pathways for site-specific drug delivery appears to be maturing into a rational approach which merits serious consideration in designing new chemo-therapeutic agents as well as resurrecting toxicologically unusable but otherwise effective molecules for a wide variety of disorders. Clinical applications of these principles are likely to occur faster in the cases where cells are of macrophage lineage or hepatocytes because detailed characteristics of efficient receptor systems largely limited to these cell types are known. Ability to deliver drugs even to these two cell types would have a major impact in the therapy of a large number of disease states—infectious, metabolic and neoplastic.

We have attempted to focus this discussion to the task of selective drug delivery to macrophages using soluble macromolecular drug conjugates. Three receptor systems present primarily on macrophages, viz. mannosyl/fucosyl, galactosyl particle and scavenger receptors appear to provide promising routes for intracellular drug delivery to these cells. We have opted to work on scavenger receptor mediated drug delivery using MBSA as a drug carrier because of simplicity of preparation, longer shelf life and ease of formulation into apyrogenic and sterile preparations. Although not encountered in our studies so far, the possibility of adverse reactions due to immunogenicity of MBSA can be circumvented using the wide variety of ligands recognized by the scavenger receptors. Scavenger receptor mediated delivery of appropriate agents to macrophages also offers exciting opportunities to modulate the metabolism of these pivotal cells in the immune system for a variety of purposes such as suppression or enhancement of immune responses, activation of macrophages etc.

Notwithstanding the promising results, the actual availability of drugs based on the targeting principles mentioned have many hurdles to cross. Extensive pharmacokinetic and pharmacodynamic studies would need to be done using various routes of administration as well as cost considerations are to be assessed before drugs based on the elegant principles of receptor-mediated endocytosis illustrated herein would find their way to the market-place.

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