Contents

1 Introduction 1

2 Review of literature 3
   2.1 Receptor mediated endocytosis — an overview 3
   2.2 Selective delivery of drugs to target cells 4
      2.2.1 Liposome as drug carrier 5
      2.2.2 Antibody mediated drug delivery 6
      2.2.3 Receptor-mediated delivery of drugs or other agents 9
   2.3 Choice of a target cell — macrophage 13
   2.4 Choice of a receptor system 13
   2.5 Choice of a disease — Macrophage neoplasia 18
   2.6 Multidrug resistance in cancer chemotherapy 20

3 Aims and Objectives 22

4 Materials and Methods 23
   4.1 Materials 23
      4.1.1 Chemicals 23
      4.1.2 Cell lines 24
      4.1.3 Animals 24
   4.2 Methods 24
      4.2.1 Culture of different cell lines 24
      4.2.2 Freezing of cells 25
      4.2.3 Maleylation of Bovine Serum Albumin 25
CONTENTS

4.2.4 Estimation of protein .............................................. 26
4.2.5 Electrophoretic mobilities of proteins .............................. 26
4.2.6 Preparation of MBSA-DNM drug conjugate ...................... 26
4.2.7 Radioiodination of MBSA-DNM ...................................... 27
4.2.8 Estimation of cellular protein ........................................ 28
4.2.9 Assay of binding of $^{125}$I-MBSA-DNM by J774A.1 cells at 4°C 28
4.2.10 Assay of uptake and degradation of $^{125}$I-MBSA-DNM at 37°C. 29
4.2.11 Internalization of bound $^{125}$I-MBSA-DNM by J774A.1 cells . 30
4.2.12 Development of drug resistant J774A.1 cells ................. 30
4.2.13 In vitro cytotoxic activity of MBSA-DNM and free DNM ... 31
4.2.14 Intravenous injection of $^{125}$I-MBSA-DNM to determine blood clearance ....................................................... 32
4.2.15 Tissue distribution of $^{125}$I-MBSA-DNM injected intravenously into BALB/C mice ................................................. 32
4.2.16 Development of J774A.1 tumor in BALB/C mice .............. 32
4.2.17 Treatment of intraperitoneal and subcutaneous tumor with MBSA-DNM or DNM ....................................................... 33
4.2.18 Treatment of preformed subcutaneous tumor with free and conjugated daunomycin ................................................. 33
4.2.19 In vivo treatment of subcutaneous tumor with single dose .. 34
4.2.20 Evaluation of tumor weight ........................................... 34
4.2.21 Immunogenicity of MBSA-DNM, DNM and MBSA ........... 34
4.2.22 Enzyme Linked Immunosorbent Assay (ELISA) ............... 34

5 Results ................................................................. 36
5.1 Characterization of MBSA-DNM drug conjugate .................... 36
5.2 Binding of $^{125}$I-MBSA-DNM by J774A.1 Cells at 4°C ............ 39
5.3 Accumulation and degradation of $^{125}$I-MBSA-DNM by J774A.1 cells at 37°C ................................................................. 39
5.4 Internalization of bound $^{125}$I-MBSA-DNM by J774A.1 cells .... 45
5.5 Competition of degradation of radiolabelled drug conjugate .... 45
5.6 Competition of degradation of $^{125}$I-MBSA-DNM by ligands recognized by the scavenger receptors ........................................... 49  
5.7 Specificity of the drug conjugate .......................................................... 49  
5.8 *In vitro* Cytotoxic activity of MBSA-DNM and free DNM ..................... 52  
5.9 Ability of drug treated cells to form monolayer .................................... 54  
5.10 Comparative effect of MBSA-DNM and DNM on $^3$H-thymidine uptake by J774A.1 and Bowes Melanoma cells ................................. 54  
5.11 Ability of drug treated cells to form tumor in mice ............................... 56  
5.12 Development of multidrug resistant cell ............................................ 56  
5.13 Restoration of cytotoxic activity of different drugs on JD-100 cells .......... 60  
5.14 Status of scavenger receptor on J774A.1 and JD-100 cells .................... 61  
5.15 Internalization of MBSA-DNM by JD-100 cells .................................. 64  
5.16 Cytotoxic activity of MBSA-DNM and DNM on JD-100 cells .................. 64  
5.17 Fate of the drug conjugate in BALB/C mice ....................................... 64  
5.18 *In Vivo* antitumor efficacy of MBSA-DNM ........................................ 68  
5.19 Time course of treatment of subcutaneous tumor of BALB/C mice with MBSA-DNM and DNM. .................................................. 68  
5.20 Treatment of established subcutaneous tumor in BALB/C mice .............. 73  
5.21 Survival of tumor bearing BALB/C mice treated with MBSA-DNM and free DNM .................................................. 77  
5.22 Immunological response evoked after drug therapy ............................. 77

6 Discussion ......................................................................................... 79
6.1 Construction of the selective drug delivery system .............................. 79
6.2 Kinetics of uptake and degradation of drug conjugate by J774A.1 cells .... 80
6.3 Status of scavenger receptor on multidrug resistant tumor cells .......... 82
6.4 Cytotoxic activity of MBSA-DNM: *in vitro* studies ............................. 83
6.5 *In vivo* antitumor efficacy of MBSA-DNM over free DNM .................... 85

7 Summary ......................................................................................... 87

8 Bibliography .................................................................................... 89