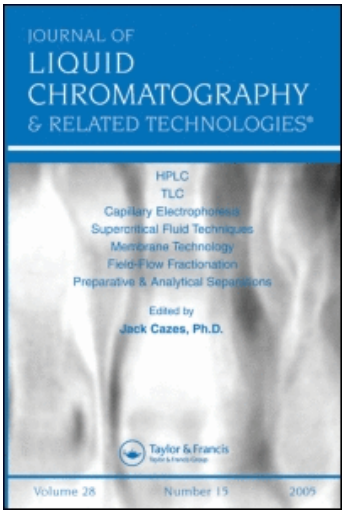


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# STUDY OF DNA INTERACTIONS WITH MELPHALAN, BUSULPHAN, AND ANALOGUES USING AN HPLC METHOD

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## ABSTRACT

A simple reversed phase HPLC method suitable to study the interactions of alkylating agents with DNA is presented in this paper. DNA interaction is expressed as the % DNA peak size exclusion. The effects caused by the antitumor drugs melphalan, busulphan, and busulpan analogues on DNA were clearly observed through chromatographic data. The synthetic dimethanesulphonates of 2-tetradecyl-1,4-butanediol and 1,2-hexadecanediol were proved more potent than busulphan.

## INTRODUCTION

Many chemotherapeutic drugs used in the treatment of cancer are bifunctional and able to crosslink biological macromolecules.[1] The most important cellular target is believed to be DNA and in the case of alkylating agents the for-

mation of interstrand crosslinks may be the most relevant cytotoxic lesion. Crosslinking starts by an initial covalent reaction of drugs with an electrophilic site on the DNA to form a monoadduct, followed by a second reaction with the other DNA strand.

Members of the homologous series of alkanediol dimethanesulphonates of general formula $CH_3SO_2O(CH_2)_nOSO_2CH_3$ have been studied for the ability to produce DNA interstrand crosslinking and DNA sequence selectivity of guanine N-7 alkylation.[2] The best known agent of this series busulphan (n=4) (**1**) (Figure 1) is one of the drugs of choice in the treatment of chronic myeloid leukemia.[3] It was suggested that busulphan produced crosslinking in DNA through a GG bridge, but no distinction could be made as to whether this was derived from inter- or intrastrand crosslinks.[4,5]

Melphalan (**2**) (Figure 1), a bis(2-chloroethyl)methylamine derivative, is another clinically useful cancer chemotherapy drug, which causes a similar effect on DNA. Its mechanism of action involves binding in the minor groove of DNA and predominant reaction at the N-7 position of guanine.[6,7]



Several physicochemical techniques for the measurement of DNA crosslinking exist.[8] However, these methods are, in many cases, insensitive, time consuming, and may require large amounts of DNA and testing material. In the present work, the interactions of the alkylating drugs busulphan and melphalan, as well as, two new lipidic busulphan analogues, dimethanesulphonates of 2-tetradecyl-1,4-butanediol (**3**) and 1,2-hexadecanediol (**4**) (Figure 1), with DNA were studied *in vitro* using an HPLC reversed phase method. This method has been used for the study of DNA interactions (ionic interactions, intercala-

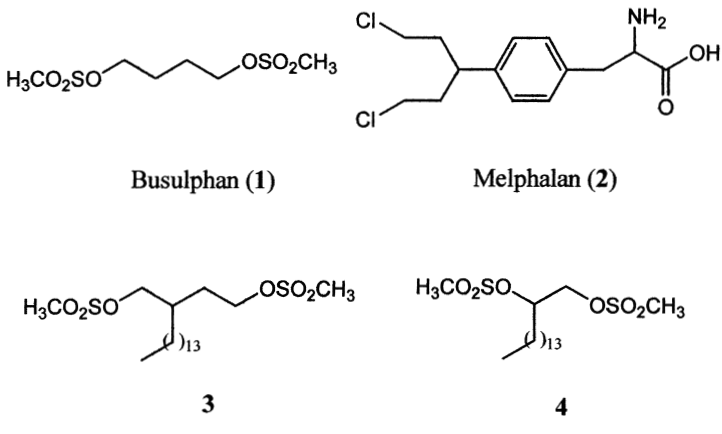


**Figure 1**. Structures of compounds tested.

tion) with some biomolecules and it is characterized by simplicity and reproducibility.[9-12] Additionally, it has shown good correlation with brine shrimp toxicity and crown gall tumor inhibition tests.[13]

## EXPERIMENTAL

### Materials

Calf thymus DNA type XV, prepared by Aposhian and Kornberg method,[14] was purchased from Sigma Chemical Co (St. Louis, MO). DNA solutions were prepared in water (0.1 mg/mL) and kept at 4°C. Busulphan and melphalan were purchased from Aldrich. Lipidic busulphan analogues **3** and **4** were prepared by treatment of 2-tetradecyl-1,4-butanediol and 1,2-hexadecanediol, respectively, with methanesulphonyl chloride in the presence of triethylamine. Details on their synthesis and their *in vitro* cytotoxicity against various cancer cell lines will be published elsewhere. HPLC grade methanol was obtained from Lab Scan. All solvents and solutions for HPLC analysis were filtered through 0.22 μm Millipore membranes before injection. Mobile phase was degassed with ultrasonic prior to use.



### Apparatus and Chromatography

A Hewlett-Packard model 1050 HPLC (Palo Alto, CA, USA), isocratic pump and a Rheodyne injector Model 7125 fitted with a 20 μL loop were used. A line source detector at 254 nm was used, while signals were recorded on a Hewlett-Packard Model HP3395 integrator. The column was a 4x250 mm, 5 μm octadecylsilane column (Lichrospher RP-18). The column was equilibrated with a $H_2O$:MeOH (80:20, v/v) mixture. Test samples and DNA solutions were introduced in a ratio 1:1 (v/v) into the sample loop (20 μL) after the appropriate incubation. The flow rate was maintained at 1 mL/min. The free DNA eluted from the column in approximately 1 min. After each experiment the column was washed with methanol for 20 min.

All compounds were tested in triplicate.

## RESULTS AND DISCUSSION

The appropriate incubation conditions (concentration, time, and temperature) in order to observe, by HPLC, the interactions of DNA with the compounds under investigation were determined. The reduction of DNA peak size was used for the measurement of these interactions. The results obtained for all the compounds tested, along with the incubation conditions, are summarized in Table 1. DNA, by itself, was tested and found to be stable under all the incubation conditions used in this study.

**Table 1**

**Effect Caused by Melphalan, Busulphan, and Analogues on DNA**

| Compound (Concentration) | Incubation Conditions Time (h) | Temperature (°C) | % DNA Peak |
|---|---|---|---|
| **DNA** | | | |
| (0.05 mg/mL) | 0.5 | 25 | 100 |
| (0.05 mg/mL) | 1.5 | 25 | 100 |
| (0.05 mg/mL) | 3 | 25 | 100 |
| 0.05 mg/mL) | 6 | 37 | 100 |
| **DNA + Melphalan** | | | |
| (0.05 mg/mL + 0.25 mg/mL) | 0.5 | 25 | 85 ± 4 |
| (0.05 mg/mL + 0.25 mg/mL) | 1.5 | 25 | 67 ± 3 |
| (0.05 mg/mL + 0.25 mg/mL) | 3 | 25 | 39 ± 4 |
| **DNA + Busulphan** | | | |
| (0.05 mg/mL + 0.25 mg/mL) | 3 | 25 | 100 |
| (0.05 mg/mL + 0.25 mg/mL) | 6 | 37 | 88 ± 5 |
| (0.05 mg/mL + 0.50 mg/mL) | 6 | 37 | 68 ± 3 |
| **DNA + Compound 3** | | | |
| (0.05 mg/mL + 0.50 mg/mL) | 6 | 37 | 0 |
| **DNA + Compound 4** | | | |
| (0.05 mg/mL + 0.50 mg/mL) | 6 | 37 | 0 |



Melphalan was incubated with DNA at a final concentration of 0.25 mg/mL at room temperature. Under these conditions the incubation time seems to play an important role in the formation of DNA crosslinks, affecting the reduction of the DNA peak, as is clearly demonstrated in Figure 2. After 3 h of incubation a considerable amount of DNA has been attacked by melphalan and the DNA peak has been reduced to 39 ± 4%.

Busulphan appears to present a weaker effect on DNA; longer incubation period time and higher drug concentration are needed to observe an effect by HPLC. After incubation at 37°C for 6 h, the DNA peak was reduced to 88 ± 5% and 68 ± 3%, when the concentration of busulphan was 0.25 mg/mL and 0.50 mg/mL, respectively.

The results obtained for melphalan and busulphan reveal a significant difference between the mode of action of these antitumor drugs to DNA. The nitrogen mustard melphalan is an efficient crosslinker. On the contrary, busul-

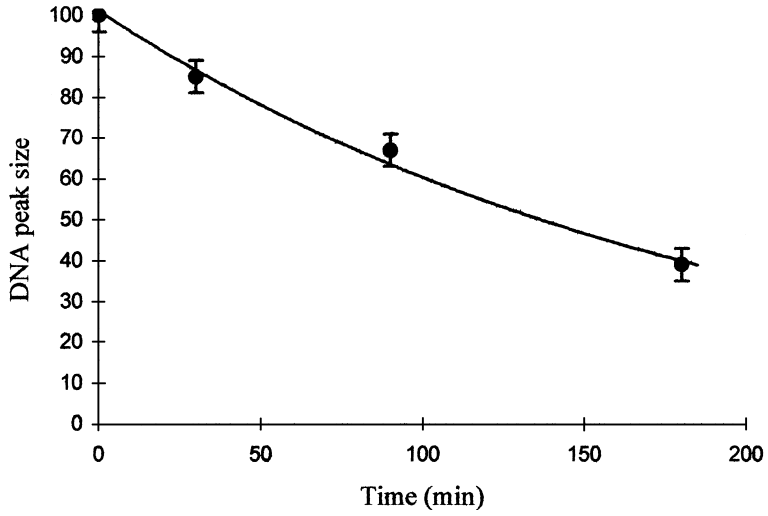


**Figure 2**. Reduction of DNA peak size during incubation with melphalan. DNA (0.05 mg/mL) and melphalan (0.25 mg/mL) were incubated at 25°C.



phan acts slowly causing low levels of crosslinks, which are increased at relatively high concentrations. These observations, obtained by the present HPLC method, are in full agreement with the results reported by other methods.[2,8]

The busulphan analogues **3** and **4** were incubated with DNA, at a concentration of 0.50 mg/mL, for 6 h at 37°C. A strong effect on DNA (complete DNA peak elimination) was observed by both analogues, which appeared more active than busulphan. It is obvious that the presence of the long aliphatic chain in synthetic analogues **3** and **4** facilitates the molecular interactions with DNA. Furthermore, the presence of the long chain confers lipophilicity to these analogues that may facilitate transport across the cellular membrane and increase intracellular drug accumulation, thus, improving drug effectiveness.

## CONCLUSIONS

The present *in vitro* study concerning DNA interactions with the antitumor drugs melphalan and busulphan and the busulphan analogues has been carried out for the first time by a simple reversed phase HPLC method. The effects caused by these alkylating agents on DNA at various conditions (incubation time, temperature) were clearly observed. The synthetic dimethanesulphonates of 2-tetradecyl-1,4-butanediol and 1,2-hexadecanediol were proven more potent than busulphan.

## REFERENCES

1. K. W. Kohn, **Molecular Actions and Targets for Cancer Chemotherapeutic Agents**, Sartorelli, Lazo, Bertino, eds., Academic Press, New York, 1981, pp 3-16.

2. M. Ponti, R. L. Souhami, B. W. Fox, J. A. Hartley, Br. J. Cancer, **63**, 743-747 (1991).

3. D. A. G. Galton, Lancet, **246**, 208-213 (1953).

4. P. Brookes, P. D. Lawley, Biochem. J., **80**, 496-503 (1961).

5. W. P. Tong, D. P. Ludlum, Biochim. Biophys. Acta, **608**, 174-181 (1980).

6. J. A. Hartley, S. M. Forrow, R. L. Souhami, Biochemistry, **29**, 2985-2991 (1990).

7. B. L.Smith, G. B. Bauer, L. F. Povirk, J. Biol. Chem., **269**, 30587-30594 (1994).

8. J. A. Hartley, M. D. Berardini, R. L. Souhami, Anal. Biochem., **193**, 131-134 (1991).

9. G. A. Karikas, V. Constantinou-Kokotou, G. Kokotos, J. Liq. Chrom. Rel. Technol., **20**, 1789-1796 (1997).

10. G. A. Karikas, K. H. Schulpis, G. Kokotos, T. Michas, S. Georgala, Clin. Biochem., **30**, 439-442 (1997).

11. K. H. Schulpis, G. A. Karikas, G. Kokotos, Clin. Chem., **44**, 178-179 (1998)

12. G. A. Karikas, K. H. Schulpis, G. Reclos, G. Kokotos, Clin. Biochem., **31**, 405-407 (1998).

13. M. P. Gupta, A. Monge, G. A. Karikas, A. Lopez de Cerain, P. N. Solis, E. De Leon, Trujillo, O. Suarez, F. Wilson, G. Montenegro, Y. Noriega, A. I. Santana, M. Correa, C. Sanchez, Int. J. Pharmacog., **34**, 19-27 (1996).

14. H. V. Aposhian, A. Kornberg, J. Biol. Chem., **237**, 519-525 (1962).

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