International Journal of Pharmaceutics, 68 (1991) 151–156

ADONIS 037851739100084D

IJP 02296

Permeability of latex membranes to anti-cancer drugs

Robert M. Mader, Blanka Rizovski, Günther G. Steger, Kurt Moser, Hugo Rainer and Christian Dittrich

Department of Chemotherapy, University of Vienna, Lazarettgasse 14, A-1090 Vienna (Austria)

(Received 3 August 1990)
(Accepted 29 August 1990)

Key words: Anti-cancer drug; Latex membrane; Permeability

Summary

The question as to whether latex membranes are pervious to anti-cancer drugs or not is closely associated with the risk during the manipulation of cytostatic drugs with latex gloves. We investigated the permeability of thick latex gloves (Sempermed Protector™) to 12 widely used anti-cancer drugs in dialysis experiments and analysed the dialysate by high-performance liquid chromatography or by capillary gas chromatography. Methotrexate, arabinofuranosylcytosine (Ara-C), 5-fluorouracil, adriamycin, mitoxantrone, 4-*O'*-tetrahydropyranyladriamycin (THP), vincristine, etoposide and cisplatin were not detectable in the dialysate after 4 h. In some of the experiments cyclophosphamide and bleomycin were detected after 4 h, but not after 2 h. 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) and mitoxantrone were found to permeate the latex membrane within 15 min. The dialysate of the BCNU original drug formulation (3.3 mg/ml) reached a maximum concentration of 0.569 mg/ml; the maximum concentration of mitoxantrone was three orders of magnitude lower.

Introduction

While numerous investigations concern the avoidance of toxic side effects in patients under cytostatic chemotherapy, less attention was paid to the safety of the oncologic staff handling anti-cancer drugs daily. During the manipulation of cytostatic agents inhalation and resorption through the skin are possible routes of uptake. While the inhalation of anti-cancer drug containing aerosols is avoided by the manipulation of drug formulations in laminar air flows, no investigation was performed to prove the protective effect of latex gloves after drug contamination. Taking into account the high distribution coefficient of some anti-cancer agents between organic solvents and buffer solutions, it is quite possible that lipophilic molecules are able to diffuse rapidly through latex membranes. Some investigations revealed mutagenicity of urine samples from oncologic nurses after manipulation of cytostatic agents (Falck et al., 1979; NGuyen et al., 1982; Benhamou et al., 1986; Poyen et al., 1988). Chromosome aberrations in lymphocytes (Waksvik et al., 1981; Nikula et al., 1984; Pohlova et al., 1986) were not confirmed in all investigations (Stiller et al., 1983) indicating exposure only after incautious handling of these drugs. Malformations in the offspring of oncologic nurses (Hemminki et al., 1985) and spontaneous abortions (Selevan et al., 1985) might

Correspondence: R.M. Mader, Dept of Chemotherapy, University of Vienna, Lazarettgasse 14, A-1090 Vienna, Austria.

be possible consequences. Liver damage induced by cytostatic drugs is first indicated by a decrease in microsomal enzyme activity and seems to lead to irreversible fibrosis (Sotanieni et al., 1983). Although several guidelines for the safe use of antineoplastic drug formulations have been published (Hoffman, 1980; Knowles and Virden, 1980; Zimmermann et al., 1981; D'Arcy, 1983; Bingham, 1985), the problem has not been solved yet (Evelo et al., 1986).

The aim of this study was therefore the examination of the permeability of thick latex gloves (Sempermed Protector™), especially designed for the manipulation of anti-cancer drugs.

Materials and Methods

Fingers from latex gloves (Sempermed Protector™ from Semperit, Austria) were used as a dialysis membrane against isotonic solutions of 12 frequently applied anti-cancer agents after rinsing them with cold water. One finger of the glove was filled with 0.9% saline and was immersed into a

TABLE 1

Investigated anti-cancer drugs

Generic name Trade Mark (Manufacturer)	Original concentration (mg/ml)	Dilution
Alkylating agents		
1,3-Bis(2-chloroethyl)-1-nitrosourea (= BCNU)		
BiCNU Carmustine (Bristol-Myers, U.S.A.)	3.3	1:20
Cyclophosphamide		
Endoxan (Asta, Germany)	20	1:100
Antimetabolites		
4-Amino-10-methyl-folic acid (= methotrexate)		
Methotrexat 'Lederle' (Cyanamid, Germany)	25	1:50
1-β-D-Arabinofuranosylcytosine (= Ara-C)		
Alexan (Mack-Illertissen, Germany)	20	1:50
5-Fluorouracil		
Fluoro-uracil (Roche, Switzerland)	25	1:50
Antibiotics		
Adriamycin		
Adriblastin (Farmitalia, Switzerland)	2	1:10
Bleomycin sulfate		
Bleomycin (Lundbeck, Denmark)	1.5	1:10
Mitoxantrone		
Novantron (Cyanamid, Germany)	2	1:100
4-*O'*-Tetrahydropyranyladriamycin (= THP)		
Pirarubicin (Behring, Germany)	2	1:10
Vincaalkaloids		
Vincristinsulfate		
Oncovin (Lilly, Germany)	1	1:10
Other cytostatic drugs		
4-*O*-Demethyl-1-*O*-(4,6-*O*-ethyliden-β-glucopyranosyl)epipodo-phyllotoxin [a] (= Etoposide)		
Vepesid (Bristol-Myers, Germany)	10	1:25
cis-Diamminedichloroplatinum (= cisplatin)		
Platinol (Bristol-Caribbean, Puerto Rico)	1	1:10

[a] The original drug formulation of Vepesid (20 mg/ml) was diluted 1:2 with 0.9% saline because of the high viscosity of the solvent polyethyleneglycol 300.

gently stirred solution of the cytostatic drug. Samples were drawn from inside the glove immediately before (negative control) and 15, 30, 60, 120 and 240 min after the beginning of the dialysis. Under light protection and at room temperature, two different concentrations were assayed in duplicates (original drug formulation and a dilution between 1 : 10 and 1 : 100 in 0.9% saline corresponding to the concentration clinically used for infusion).

BCNU is unstable at room temperature (an approximative loss of 6% for a period of 3 h is indicated by the manufacturer). Precipitation in the original drug formulation at 4°C prevented dialysis in the cold, where stability is indicated for 24 h. The concentration of 3.3 mg/ml was therefore assayed at room temperature, whereas the dilution was assayed at 4°C.

The evaluated anti-cancer drugs are listed in Table 1.

Analytical methods

HPLC

All anti-cancer drugs except cyclophosphamide were evaluated by HPLC (Hewlett Packard, U.S.A., model 1090 M equipped with a diode array detector). Fluorescence was monitored instead of absorption for the anthracyclines adriamycin and THP (fluorescence detector Shimadzu, Japan, model RF-530 with integrator Hewlett Packard, U.S.A., model 3392A).

A 20 μl aliquot of each sample was injected onto HPLC after centrifugation without further sample purification and was analysed according to the conditions listed in Table 2.

Derivatisation of cisplatin 50 μl of an aqueous sample containing cisplatin was derivatised with 10 μl of 10% DDTC (diethyldithiocarbamate, Sigma, U.S.A., Code D-3506) in 0.1 N NaOH (w/v) at 37°C for 30 min. After 5 min in an ice-bath, 100 μl chloroform was added and the sample was vortexed for the same time period. After centrifugation, the organic layer was directly injected onto HPLC (Andrews et al., 1984).

Gas chromatography

Cyclophosphamide was evaluated by capillary gas chromatography (Hewlett Packard, U.S.A., model 5890) on an HP-5 column (methyl phenyl silicone gum from Hewlett Packard, U.S.A.), di-

TABLE 2

Conditions for the analysis by HPLC

Anti-cancer drug	Column	Eluent			Flow rate (ml/min)	Detection
		% Buffer	% ACN	% MeOH		
BCNU	A	50	50	–	0.8	UV, 231 nm
Methotrexate	A	90	10	–	0.8	UV, 300 nm
Ara-C	A	100	–	–	0.8	UV, 279 nm
5-Fluorouracil	A	100	–	–	0.8	UV, 265 nm
Adriamycin	A	65	35	–	1.2	fluorescence ex = 478 nm, em = 552 nm
Bleomycin	A	77	23	–	0.8	UV, 292 nm
Mitoxantrone	A	75	25	–	1.2	Vis, 600 nm
THP	A	65	35	–	1.2	fluorescence ex = 478 nm, em = 552 nm
Vincristine	A	35	65	–	0.8	UV, 296 nm
Etoposide	A	65	35	–	0.8	UV, 285 nm
Cisplatin [a]	B	30	–	70	0.8	UV, 256 nm

[a] Water (HPLC-grade obtained from Rathburn, U.K., Code 1020) was used instead of buffer solution for the elution of the platinum-diethyl-dithiocarbamate complex.

Columns: A, μ-Bondapak Phenyl, 10 μm particles, dimensions 300 × 3.9 mm (Waters Chromatography Division, U.S.A.); B, Versapak-1, C_{18}, 10 μm particles, dimensions 250 × 4 mm (stationary phase supplied from Alltech, U.S.A., filled at the research centre Seibersdorf, Austria). *Eluents:* Buffer, 35 mM ammonium formate buffer + 1 mM EDTA (Sigma, U.S.A., Code ED4SS), pH 3; ACN, acetonitrile Promochem Chrom AR (Mallinckrodt, U.S.A., Code 2856); MeOH, methanol Promochem Chrom AR (Mallinckrodt, U.S.A., Code 3041).

mensions 25 m × 0.2 mm i.d. × 0.33 μm film thickness as stationary phase. The injector was set to 250°C, the nitrogen-phosphorus detector to 270°C. After 1 min at 130°C the temperature was raised to a final value of 270°C at a gradient of 10°C/min. The gas flow settings were: carrier helium, 1 ml/min; auxiliary gas helium, 30 ml/min; hydrogen, 3.5 ml/min; air, 100 ml/min. 1 μl sample was injected splitless.

Results

According to their diffusion velocity we divided the assayed anti-cancer drugs in three different groups: non penetrating drugs, slowly penetrating drugs and rapidly penetrating drugs. Eight of the evaluated cystostatic drugs were not detectable after a dialysis of 4 h:

Group A: Non-Penetrating Drugs	*Detection limit*
Methotrexate	0.75 ng
Arabinosyl-cytosine	1 ng
5-Fluorouracil	1.4 ng
Adriamycin	0.2 ng
4-*O'*-Tetrahydropyranyladriamycin	0.8 ng
Vincristine	23 ng
Etoposide	20 ng
Cisplatin	100 ng

Cyclophosphamide and bleomycin were detected in some experiments after 4 h, but never after 2 h of dialysis:

Group B: Slowly Penetrating Drugs	*Highest concentration found*
Cyclophosphamide	2.4 ppm
Bleomycin	4.1 μg/ml dialysate

BCNU and mitoxantrone were found to diffuse rapidly into the gloves:

Group C: Rapidly Penetrating Drugs	*Highest concentration found*
BCNU	569 μg/ml dialysate
Mitoxantrone	0.26 μg/ml dialysate

Discussion

The investigation focused on the most widely used drugs for the therapy of solid tumours with some exceptions motivated by interesting molecular structures such as the adriamycin analogue THP, which was assayed because of its higher lipophilicity and the anthracenedione mitoxantrone, which is structurally unique. BCNU is a very small lipophilic compound designed to cross the blood-brain barrier and is useful in the treatment of neurological tumours.

Three factors essentially influence the diffusion of drugs through biological membranes: pH value (ionisation of the drug), lipophilicity and molecular weight (size of the drug). Most of the cytostatic drugs in group A were expected not to permeate through the extremely lipophilic latex membrane. Ionised drugs such as methotrexate, highly water-soluble drugs, e.g. 5-fluorouracil and Ara-C, and very polar substances such as cisplatin are generally excluded from such processes as a synthetic membrane allows only passive diffusion and convective transport with the solvent. Adriamycin (partition coefficient $P = 0.79$ in the system octanol/water; this and the following partition coefficients are cited from Lavin (1980) and Workman (1986)), its analogue THP (P not known, but expected to be somewhat higher), the very lipophilic drugs vincristine ($P = 631$) and etoposide (P not known, but estimated to be similar), have a molecular weight of more than 400 which is an accepted limit for convective transport through biological membranes (Ritschel, 1986). Although a biological membrane and a latex membrane share few common attributes, these observations made for biological membranes are also applicable to the latex membrane, which has nearly no convective transport with the solvent water.

The results obtained for the drugs in group B could be explained only partially. Bleomycin is a hydrophilic substance ($P = 0.0005$) and its molecular weight of 1400 should inhibit diffusion. Cyclophosphamide as a substance with inter-

mediate size and lipophilicity was at least suspected to cross the membrane, but could be detected in the dialysate only once. The low molecular weight and the lipophilicity of BCNU (MW = 214, $P = 34.7$) explain the high diffusion velocity and the extraordinarily high concentration found in our experiments (Fig. 1, original formulation of BiCNU carmustine). Latex is pervious to BCNU which diffuses rapidly into the gloves with a delay of some minutes after contamination with the drug. Analogues of BCNU such as CCNU (MW = 234, $P = 676$) are suspected to behave in a similar manner.

Independent of the drug concentration of the solution to be dialysed, very similar diffusion kinetics were observed for mitoxantrone (group C), as shown in Fig. 2. Compared with BCNU the concentration of mitoxantrone inside the gloves was three orders of magnitude lower, due to a partially charged molecule with an intermediate molecular weight of 517 and a very lipophilic ring system.

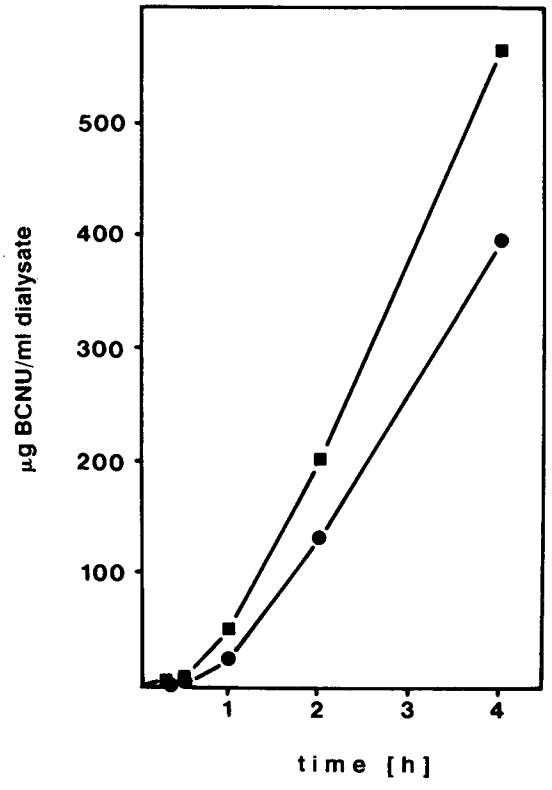

Fig. 1. Diffusion of BCNU into latex gloves. The concentration of BCNU was 3.3 mg/ml in the dialysed solution in both experiments.

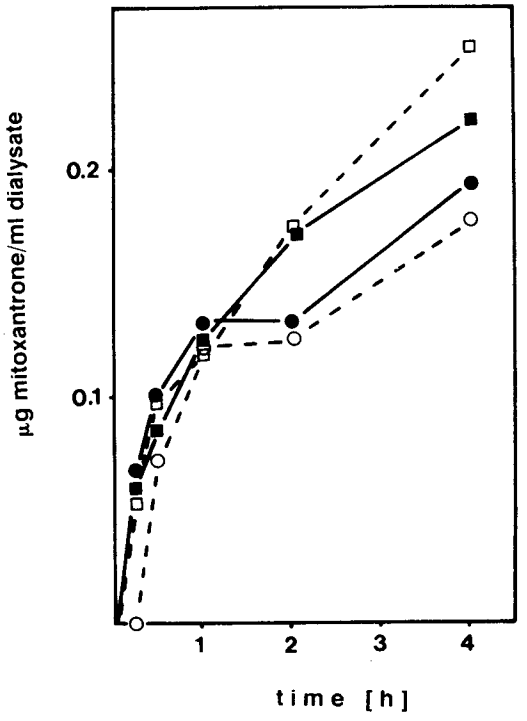

Fig. 2. Diffusion of mitoxantrone into latex gloves. The concentration of mitoxantrone was 2 mg/ml [(●———●) experiment 1, (■———■) experiment 2] and 20 µg/ml [(○- - - - -○) experiment 3, (□- - - - -□) experiment 4] in the dialysed solution.

We conclude that for the vast majority of anticancer drugs thick latex gloves (Sempermed Protector™) represent a very good protection against transdermal drug delivery when continuously used for no longer than 2 h. Only for BCNU and mitoxantrone are systemic toxic effects to be expected in case of contamination requiring special care during the handling of these two substances.

Acknowledgement

This investigation was. in part supported by 'Kommission Onkologie (Krebsforschung) der Medizinischen Fakultät der Universität Wien'.

References

Andrews, P.A., Wung, W.E. and Howell, S.B., A high-performance liquid chromatographic assay with improved selectivity for cisplatin and active platinum (II) complexes in plasma ultrafiltrate. *Anal. Biochem.*, 143 (1984) 46–56.

Benhamou, S., Callais, F., Sancho-Garnier, H., Min, S., Courtois, Y.A. and Festy, B., Mutagenicity in urine from nurses handling cytostatic agents. *Eur. J. Cancer Clin. Oncol.*, 22 (1986) 1489–1493.

Bingham, E., Hazards to health workers from antineoplastic drugs. *N. Engl. J. Med.*, 313 (1985) 1220–1221.

D'Arcy, P.F., Reactions and interactions in handling anticancer drugs. *Drug Intell. Clin. Pharm.*, 17 (1983) 532–538.

Evelo, C.T.A., Bos, R.P., Peters, J.G.P. and Henderson, P.T., Urinary cyclophosphamide assay as a method for biological monitoring of occupational exposure to cyclophosphamide. *Int. Arch. Occup. Environ. Health*, 58 (1986) 151–155.

Falck, K., Gröhn, P., Sorsa, M., Vainio, H., Heinonen, E. and Holsti, L.R., Mutagenicity of urine of nurses handling cytostatic drugs. *Lancet*, i (1979) 1250–1251.

Hemminki, K., Kyyronen, P. and Lindbohm, M.-L., Spontaneous abortions and malformations in the offspring of nurses exposed to anaesthetic gases, cytostatic drugs and other potential hazards in hospitals based on registered information of outcome. *J. Epidemiol. Community Health*, 39 (1985) 141–147.

Hoffmann, D.M., The handling of antineoplastic drugs in a major cancer center. *Hosp. Pharm.*, 15 (1980) 302–304.

Knowles, R.S. and Virden, J.E., Handling of injectable antineoplastic agents. *Br. Med. J.*, 281 (1980) 589–591.

Lavin, V.A., Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability. *J. Med. Chem.*, 23 (1980) 682–684.

NGuyen, T.V., Theiss, J.C. and Martney, T.S., Exposure of pharmacy personnel to mutagenic antineoplastic drugs. *Cancer Res.*, 42 (1982) 4792–4796.

Nikula, E., Kiviniitty, K., Leisti, J. and Taskinen P.J., Chromosome aberrations in lymphocytes of nurses handling cytostatic agents. *Scand. J. Work Environ. Health*, 10 (1984) 71–74.

Pohlova, H., Cerna, M. and Rossner, P., Chromosomal aberrations sister-chromatid exchange and urine mutagenicity in workers occupationally exposed to cytostatic drugs. *Mutat. Res.*, 174 (1986) 213–218.

Poyen, D., DeMeo, M.P., Gouvernet, J. and Dumenil, G., Handling of cytostatic drugs and urine mutagenesis. *Int. Arch. Occup. Environ. Health*, 61 (1988) 183–188.

Ritschel, W.A., *Handbook of Basic Pharmacokinetics*, Drug Intelligence Publications, Hamilton, 1986, pp. 43–61.

Selevan, S.G., Lindbohm, M.-L., Hornung, R.W. and Hemminki, K., A study of occupational exposure to antineoplastic drugs and fetal loss in nurses. *N. Engl. J. Med.*, 313 (1985) 1173–1178.

Sotanieni, E.A., Sutinen, S., Arranto, A.J., Sutinen, S., Sotaniemi, K.A., Lethola J., and Pelkonen, R.O., Liver damage in nurses handling cytostatic agents. *Acta Med. Scand.*, 214 (1983) 181–190.

Stiller, A., Obe, G., Boll, I. and Pribilla, W., No elevation of the frequences of chromosomal alterations as a consequence of handling cytostatic drugs: analyses with peripheral blood and urine of hospital personnel. *Mutat. Res.*, 121 (1983) 3–4.

Waksvik, H., Klepp, O. and Brogger, A., Chromosome analyses of nurses handling cytostatic agents. *Cancer Treat. Rep.*, 65 (1981) 607–610.

Workman, P., The pharmacology of brain tumour chemotherapy. In: Bleehan, N.M. (Ed.), *Tumours of the Brain*, Springer, Berlin, 1986, pp. 183–200.

Zimmermann, P.F., Larsen, R.K., Barkley, E.W. and Gallelli, J.F., Recommendations for the safe handling of injectable antineoplastic drug products. *Am. J. Hosp. Pharm.*, 38 (1981) 1693–1695.