TEST REPORT

Permeability of two tubular foils to carmustine and thiotepa

By order of

Berner International GmbH
Mühlenkamp 6
D-25337 Elmshorn

Carried out by

Apotheker Gerhard Carstens
Dipl. Biochem. Michael Klein

St. Bernward Apotheke
Hildesheimer Straße 240
D-30519 Hannover
(+49 511 839796
Ü st.bernward.apo@pharma-online.de

Objective

The testing was conducted to determine the permeation of two cytotoxic drugs - carmustine and thiotepa - through two tubular foils provided by Berner International GmbH, Mühlenkamp 6, D-25337, Germany.

Material and Method

Tubular foils

Both tubular foils tested were made of a transparent colourless plastic material with a diameter of approx. 56 cm (in plain folded form). No more detailed information was available on chemical or physical properties of the material. The foils were delivered in a paper roll (Foil 1: rolled, with a handwritten marking "Paxxo, 071024, Till Tyskland") and in a plastic bag (Foil 2: folded, without any marking), respectively, on 13th December 2007. They were stored up to the test beginning at room temperature in a light-tight cabinet. Samples were randomly taken from the accessible end of each foil.

Cytotoxic drugs

The foil materials were stressed with carmustine and thiotepa in form of commercially available formulations. Both cytotoxic solutions comprised only one cytotoxic agent and a solvent. They were prepared from dry chemicals according to the manufacturers' recommendations using the enclosed solvent (100% ethanol) and/or distilled water. Table 1 lists the cytotoxic agent, name of the formulation, lot and expiry, as well as the concentration of the agent inside the test apparatus.

Table 1: Cytotoxic drugs

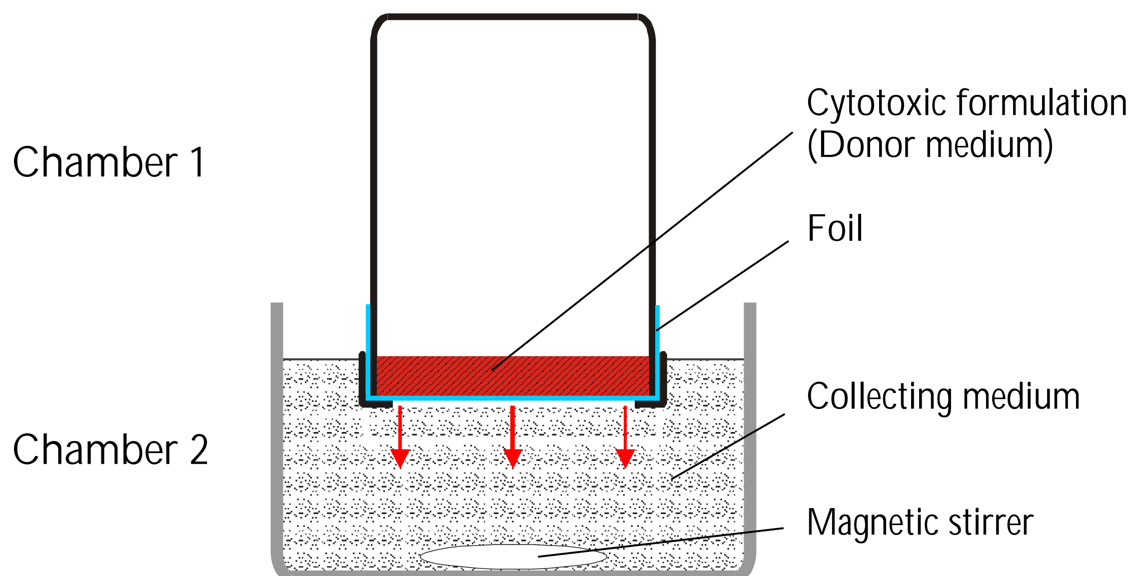
Active agent	Formulation	Manufacturer	Lot Expiry	Concentration [mg ml^{-1}]
Carmustine	CARMUBRIS	Bristol-Myers Squibb GmbH & Co. KGaA, Sapporobogen 6-8, D-80637 München, Germany	7L29423 08.2009 Ethanol: 7K32341 08.2009	3.3
Thiotepa	Thiotepa "Lederle" 15 mg	RIEMSER Arzneimittel AG, An der Wiek 7, D-17493 Greifswald-Insel Riems, Germany	701670 12.2008	10

Test apparatus

Testing was carried out in accordance with the test procedure described in the European Standard EN ISO 6529 (‚Protective clothing – Protection against chemicals – Determination of resistance of protective clothing materials to permeation by liquids and gases' German version, 2001). The test apparatus used in this examination consisted of two chambers divided by a foil sample (Figure 1). Chamber 1 was prepared by attaching the foil to the opening of a small poly-propylene container with a pierced cap (diameter of the borehole: 26 mm). The interior surface of the foil was exposed to the interior of the container. Before closing, the chamber was charged with 3 ml of the cytotoxic formulation (donor medium). Chamber 2 consisted of a glass vessel containing 25 ml of distilled water to collect the permeated agent (collecting medium). Rapid mixing of this solution was ensured by a magnetic stirrer.

The examination was carried out over a period of 7 days (10080 minutes) from 4th to 11th March 2008 (carmustine) and from 7th to 14th May 2008 (thiotepa).

Figure 1: Permeation test apparatus



Measurements

Measurements were started by dipping chamber 1 into chamber 2. At specified times (carmustine: 0, 120, 240, 480, 1440, 2880, 4320, 8640 and 10080 min; thiotepa: 0, 120, 360, 1440, 2880, 8640 and 10080 min) a 2-ml-sample was taken from each collecting medium and frozen instantaneously at -25°C. The solution was replenished with 2 ml of fresh distilled water. Interferences caused by the co-permeated solvent or by soluble ingredients released from the foil material were determined from blank tests. These tests were carried out in the same manner, but the donor medium was composed of only 10% ethanol (carmustine) or water (thiotepa). All measurements were performed in triplicate (n = 3) at ambient temperature (carmustine: 20.3 ± 1.1°C; thiotepa: 24.3 ± 0.6°C; controlled at the beginning of each sampling course).

Analysis

Concentration values for carmustine and thiotepa were determined by UV-spectrophotometry (Uvikon Spectrophotometer 930, Kontron Instruments). For calibration standards were diluted from original CARMUBRIS or Thiotepa "Lederle" formulations. The analytical parameters are given in Table 2. To adjust the concentration (*C*) of an active agent for interferences, the absorbance value of a sample was corrected with its corresponding blank value.

With the following equation permeation rates were determined for each testing interval (0 – 120 min, 120 – 240 min etc) from the adjusted concentrations:

$$P = \frac{(C_i - C_{i-1}) \cdot V_t}{(T_i - T_{i-1}) \cdot A}$$

P = permeation rate [µg min^{-1} cm^{-2}]
C_i = adjusted concentration of the active agent in the collecting medium at time T_i [µg ml^{-1}]
i = an indexing number assigned to each discrete sample, starting with i = 1 for the first sample
T_i = time elapsed beginning initial chemical contact where T_i is the time at which discrete sample i was removed [min]
V_t = total volume of the acceptor medium [ml]
A = area of the material specimen contacted [cm^{-2}]

Adjusting of *C* using blanks could lead to negative *P*-values in some cases.

Table 2: Analytical parameters

Active agent	Wavelength [nm]	Limit of detection [µg ml^{-1}]
Carmustine	229	1.13
Thiotepa	210	1.27

Results and conclusion

Under the conditions descript above (continuous contact between the cytotoxic agent and the foil over a period of 7 days at room temperature) low permeation could be detected for both carmustine and thiotepa. Tables 3 and 4 list the permeation rates for each testing interval.

Because manufacturing-methods can differ, the results should not be generalised to other materials. Additionally, extrapolation to other cytotoxic substances or formulations containing the same active agents should not be attempted. It has been shown in other studies that the composition of the solvent (including excipients) can affect the permeation of the cytotoxic agent dramatically.

Table 3: Permeation rates of carmustine

Time [min]	Foil 1		Foil 2	
	Permeation rate [µg min^{-1} cm^{-2}]	S.D. [µg min^{-1} cm^{-2}]	Permeation rate [µg min^{-1} cm^{-2}]	S.D. [µg min^{-1} cm^{-2}]
0 – 120	0.044	0.008 *	0.056	0.002
120 –240	0.061	0.005	0.074	0.004
240 – 480	0.075	0.006	0.091	0.006
480 – 1440	0.053	0.005	0.059	0.003
1440 – 2880	0.039	0.003	0.042	0.005
2880 – 4320	0.032	0.003	0.026	0.008
4320 – 8640	0.012	0.002	-0.001	0.017 *
8640 – 10080	-0.010	0.002	-0.014	0.001

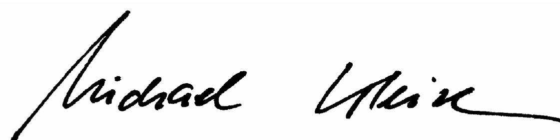
* Not detectable with regard to the limit of detection (converted to *P*)

Table 4: Permeation rates of thiotepa

Time [min]	Foil 1		Foil 2	
	Permeation rate [µg min^{-1} cm^{-2}]	S.D. [µg min^{-1} cm^{-2}]	Permeation rate [µg min^{-1} cm^{-2}]	S.D. [µg min^{-1} cm^{-2}]
0 – 120	0.027	0.003 *	0.024	0.006 *
120 –360	0.023	0.001 *	0.015	0.012 *
360 – 1440	0.024	0.002	0.026	0.003
1440 – 2880	0.026	0.002	0.025	0.002
2880 – 8640	0.032	0.003	0.031	0.003
8640 – 10080	0.039	0.002	0.038	0.006

* Not detectable with regard to the limit of detection (converted to *P*)

Hannover, 9th July 2008



Gerhard Carstens
Head of pharmacy

Michael Klein
Head of laboratory