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Selective delivery of etoposide to intraperitoneal tissues using a new dosage format: etoposide microcrystals suspended in oil

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Rats received an intraperitoneal bolus injection of etoposide at 5 mg/kg of body weight in the form of an etoposide microcrystal suspension in oil (ETOP-OIL) or an aqueous etoposide solution. The tissue distribution was subsequently analyzed using high performance liquid chromatography. ETOP-OIL delivered significantly greater amounts of etoposide and for a longer duration to the intraperitoneal tissues such as the omentum and the spleen, whereas it delivered significantly less etoposide to the rest of the body such as the lung, the heart and the bone marrow, than the aqueous etoposide solution.

Key words: Drug delivery system, drug distribution, ETOP-OIL, etoposide, intraperitoneal administration.

Introduction

Intraperitoneal administration of anticancer drugs is one of the most popular chemotherapy treatments for peritoneal carcinomatoses. This method is, however, not always effective, because intraperitoneal anticancer drugs in aqueous solutions are readily absorbed into circulating blood,[1] and are not selectively distributed at concentrated levels to the lesions located on the peritoneal surface and in the subperitoneal tissues. We have developed a new dosage format, which comprises a suspension of etoposide microcrystals in oil, for the treatment of peritoneal carcinomatoses.[2] We have already reported that this dosage format has superior therapeutic effects on peritoneal carcinomatoses in mice experiments[2] and has a reduced systemic toxicity in mice.[2] In this paper we report that this new dosage format delivers higher concentrations of etoposide selectively to the intraperitoneal tissues for a longer time while delivering lower levels of etoposide to other body tissues, as compared with an etoposide aqueous solution.

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Materials and methods

Drug preparation

Etoposide,[3] which is a podophyllotoxin derivative,[4] in the form of a microcrystalline powder, was supplied by Nippon Kayaku (Tokyo, Japan). The needle-like shaped microcrystals of etoposide were less than 0.2 mm in length, as determined by microscopic examination, and were very slightly soluble in oil according to our preliminary examination. Etoposide at 0.4 mg/ml was added to oil (iodetryl, iodinized poppy-oil used for lymphography; Lipiodol Ultra Fluid®, Laboratoires Guerbert, Paris, France). The mixture was stirred with a magnetic stirrer for 6 h forming a suspension of etoposide microcrystals in oil (ETOP-OIL).

As a control drug, a commercial aqueous etoposide solution (Lastet®, Nippon Kayaku) was diluted with water in which dimethyl sulfoxide and Tween 80 at a weight ratio of 7 to 13 were dissolved to yield an aqueous solution of 0.4 mg etoposide/ml (Etop-sol). These drugs were used within 30 min of preparation.

Drug Administration

Sixty-six male Wistar rats weighing 200 g (purchased from Shimizu Laboratory Animal Center, Kyoto, Japan) were bred under standard conditions (specific pathogen-free, room temperature of 22°C, relative humidity of 60%, day–night cycle of 12 h). The 66 rats were divided into two groups composed of 33 rats each.

Under general anesthesia with ether inhalation in order to relax the abdominal muscles, etoposide at 20 mg/kg (equal to a drug volume of 10 ml/rat) was administered intraperitoneally to the two groups in the form of ETOP-OIL or Etop-sol. Three rats from each group were sacrificed by neck breaking at 15 and 30 min, 1, 3, 6, 12 and 24 h, and on days 2, 4, 8 and 16 following drug administration. Blood samples, taken through a heart puncture up to day 16, were centrifuged at 3000 cycle/min for 5 min and the supernatants (plasma) were stored at −100°C for the etoposide assay. Tissues and organs were removed up to day 16 following etoposide administration. The omentum and the spleen were selected as representative intraperitoneal tissues. The heart, the lungs and the bone marrow of both femurs were selected as representative extraperitoneal organs. The kidney, from which etoposide is secreted into the urine,[5,6] the urinary bladder and the liver, in which etoposide is secreted into bile,[6] were selected as sample organs. Tissue samples were weighed with a microbalance and stored at −100°C for the etoposide assay.

The concentration of etoposide in the blood plasma and tissues samples was measured using high performance liquid chromatography and UV detection at a wavelength of 254 nm.[7] The assay limits were 0.1 μg etoposide/g of tissue sample and 0.04 μg etoposide/ml of plasma.

If the etoposide concentration was detectable in all of the animals' samples, the etoposide concentration was statistically compared between the two groups at each time point by the analysis of variance. Differences were considered significant if a p value of less than 0.05 was obtained.

Results

Blood plasma

The etoposide concentration in blood plasma is shown in Table 1. In the ETOP-OIL group, the concentration was 0.58 μg/ml at 15 min after administration and increased slowly to 1.15 μg/ml at 1 h, which represented the highest concentration, then gradually decreased to the undetectable range 8 days after drug administration. Using Etop-sol, the plasma concentration was 6.40 μg/ml at 15 min after administration and peaked at 13.1 μg/ml at 1 h, which was more than 10 times the plasma concentration in the ETOP-OIL group. The etoposide concentration rapidly decreased to undetectable levels by day 4. The plasma etoposide concentration in the ETOP-OIL group was significantly lower ($p < 0.025$–0.005) 15 min to 3 h after administration, and significantly higher 12 h to 2 days after administration ($p < 0.01$–0.005), compared with the Etop-sol group.

Table 1. Etoposide concentration in blood plasma

Time after administration	Etoposide concentration (mean ± SE[a] μg/ml)		Statistical significance (F-ratio)
	ETOP-OIL	Etop-sol	
15 min	0.58 ± 0.03	6.40 ± 1.02	$p < 0.005$ (32.4)
30 min	0.85 ± 0.11	9.04 ± 0.96	$p < 0.005$ (72.4)
1 h	1.15 ± 0.18	13.1 ± 5.0	$p < 0.025$ (15.2)
3 h	0.71 ± 0.05	2.26 ± 0.70	$p < 0.025$ (14.2)
6 h	0.32 ± 0.03	0.14 ± 0.04	NS
12 h	0.23 ± 0.03	0.05 ± 0.01	$p < 0.005$ (48.6)
24 h	0.12 ± 0.01	0.05 ± 0.01	$p < 0.005$ (45.1)
2 days	0.13 ± 0.01	0.05 ± 0.01	$p < 0.01$ (24.0)
4 days	0.06 ± 0.01	ND	—
8 days	ND	ND	—

[a]Mean ± SE for three experiments.
NS: not signficant.
ND: not detectable.

Intraperitoneal organs

Etoposide concentrations in the omentum (Table 2) were assayed as a measure of intraperitoneal tissue distribution. In the ETOP-OIL group, the etoposide concentration in the omentum was in the range of 168.5–86.0 μg/g during the first 2 days after administration, and 2.32 μg/g even on day 8. Conversely, in the Etop-sol group, a concentration of 94.0 μg/ml recorded at 30 min was the highest mean value achieved, and this value decreased rapidly to about 1.0 μg/g by 6 h. The concentration in the ETOP-OIL group was significantly greater from 15 min to 8 days after administration than in the Etop-sol group ($p < 0.01$–0.005).

Etoposide concentrations in the spleen were also assayed as a measure of intraperitoneal distribution (Table 3). In the ETOP-OIL group, the concentration was 76.9 μg/g at 15 min and was detectable

Table 2. Etoposide concentration in the omentum

Time after administration	Etoposide concentration (mean ± SE[a] μg/ml)		Statistical significance (*F*-ratio)
	ETOP-OIL	Etop-sol	
15 min	158.2 ± 12.8	68.5 ± 13.1	$p < 0.01$ (24.0)
30 min	168.5 ± 9.1	94.0 ± 5.9	$p < 0.005$ (47.0)
1 h	165.0 ± 19.9	36.5 ± 5.0	$p < 0.005$ (39.0)
3 h	136.1 ± 9.4	7.5 ± 1.3	$p < 0.005$ (185)
6 h	156.5 ± 7.3	0.98 ± 0.27	$p < 0.005$ (456)
12 h	86.0 ± 21.2	0.92 ± 0.11	$p < 0.025$ (16.0)
24 h	96.0 ± 16.4	1.07 ± 0.25	$p < 0.005$ (33.4)
2 days	94.5 ± 3.7	0.64 ± 0.04	$p < 0.005$ (638)
4 days	3.70 ± 0.55	0.35 ± 0.15	$p < 0.005$ (34.4)
8 days	2.32 ± 0.12	0.46 ± 0.15	$p < 0.005$ (90.3)
16 days	0.55 ± 0.02	ND	—

[a]Mean ± SE for three experiments.
NS: not signficant.
ND: not detectable.

Table 3. Etoposide concentration in the spleen

Time after administration	Etoposide concentration (mean ± SE[a] μg/ml)		Statistical significance (*F*-ratio)
	ETOP-OIL	Etop-sol	
15 min	76.9 ± 9.0	29.0 ± 5.3	$p < 0.01$ (21.3)
30 min	64.4 ± 14.3	54.6 ± 5.9	NS (0.40)
1 h	36.8 ± 2.6	15.9 ± 3.3	$p < 0.01$ (25.5)
3 h	10.5 ± 0.9	6.00 ± 1.48	NS (6.67)
6 h	5.99 ± 0.50	0.42 ± 0.06	$p < 0.005$ (122)
12 h	6.67 ± 2.24	ND	—
1 day	2.73 ± 0.43	ND	—
2 days	2.29 ± 0.17	ND	—
4 days	0.35 ± 0.15	ND	—
8 days	ND	ND	—

[a]Mean ± SE for three experiments.
NS: not signficant.
ND: not detectable.

even 4 days following administration. In the Etop-sol group, the concentration was 29.0 μg/g at 15 min, increasing to 54.6 μg/g at 30 min, which was 1.9 times the etoposide concentration noted at 15 min; it then decreased to undetectable levels at 12 h. There were statistically significant differences in etoposide concentrations between the two groups at 15 min, 1 h and 6 h ($p < 0.01$–0.005).

Extraperitoneal organs

Etoposide concentrations in the lung, heart and bone marrow were assayed as a measure of extraperitoneal organ distribution. In the heart

Table 4. Etoposide concentration in the heart

Time after administration	Etoposide concentration (mean ± SE[a] μg/ml)		Statistical significance (*F*-ratio)
	ETOP-OIL	Etop-sol	
15 min	3.02 ± 0.21	6.48 ± 0.58	$p < 0.005$ (31.2)
30 min	2.25 ± 0.69	10.58 ± 0.65	$p < 0.005$ (77.1)
1 h	1.40 ± 0.05	10.43 ± 3.14	$p < 0.05$ (8.24)
3 h	1.05 ± 0.14	3.72 ± 1.23	NS (4.64)
6 h	ND	ND	—

[a]Mean ± SE for three experiments.
NS: not signficant.
ND: not detectable.

Table 5. Etoposide concentration in the lung

Time after administration	Etoposide concentration (mean ± SE[a] μg/ml)		Statistical significance (*F*-ratio)
	ETOP-OIL	Etop-sol	
15 min	1.51 ± 0.10	6.76 ± 0.26	$p < 0.005$ (341)
30 min	5.75 ± 1.18	23.1 ± 3.7	$p < 0.025$ (19.8)
1 h	3.07 ± 0.53	15.5 ± 2.2	$p < 0.01$ (29.6)
3 h	1.30 ± 0.04	4.19 ± 1.43	NS (4.11)
6 h	0.40 ± 0.16	ND	—
12 h	0.28 ± 0.18	ND	—
1 day	ND	ND	—

[a]Mean ± SE for three experiments.
NS: not signficant.
ND: not detectable.

(Table 4), the etoposide concentrations in the ETOP-OIL group were less than 1/2 to 1/8 of that in the Etop-sol group during the first 3 h after administration. The concentration in the ETOP-OIL group was significantly lower at 15 min, 30 min and 1 h than in the Etop-sol group ($p < 0.05$–0.005).

The concentration in the lung is shown in Table 5. In the ETOP-OIL group, the etoposide concentration in the lung was less than 1/4 to 1/3 of that found in the Etop-sol group during the first 3 h after administration. The concentration in the ETOP-OIL group was significantly lower during the first 1 h than in the Etop-sol group ($p < 0.01$–0.005). The concentration in the ETOP-OIL group, however, decreased slowly and was detectable up to 12 h after administration, whereas the etoposide concentration in the Etop-sol group decreased rapidly and was undetectable at 6 h.

The concentration of etoposide in bone marrow is shown in Table 6. In the ETOP-OIL group, the concentration was less than that in the Etop-sol group at every time point and there was a significant

Table 6. Etoposide concentration in the bone marrow

Time after administration	Etoposide concentration (mean ± SE[a] μg/ml)		Statistical significance (*F*-ratio)
	ETOP-OIL	Etop-sol	
15 min	6.48 ± 0.84	7.52 ± 0.30	NS (1.35)
30 min	7.71 ± 0.36	9.34 ± 1.32	NS (1.43)
1 h	2.37 ± 0.15	4.24 ± 0.72	NS (6.41)
3 h	2.47 ± 0.05	5.83 ± 1.85	NS (3.29)
6 h	0.52 ± 0.16	1.08 ± 0.06	$p < 0.05$ (11.0)
12 h	ND	ND	—

[a]Mean ± SE for three experiments.
NS: not signficant.
ND: not detectable.

Table 8. Etoposide concentration in the urinary bladder

Time after administration	Etoposide concentration (mean ± SE[a] μg/ml)		Statistical significance (*F*-ratio)
	ETOP-OIL	Etop-sol	
15 min	16.1 ± 2.8	18.6 ± 2.84	NS (0.37)
30 min	5.91 ± 1.67	46.4 ± 11.5	$p < 0.025$ (12.1)
1 h	9.98 ± 1.30	19.6 ± 2.7	$p < 0.05$ (11.0)
3 h	2.56 ± 0.79	10.0 ± 3.0	NS (5.50)
6 h	ND	ND	—

[a]Mean ± SE for three experiments.
NS: not signficant.
ND: not detectable.

difference in the concentration between the two groups at 6 h.

Kidney and urinary bladder

The etoposide concentration in the kidney, where the drug is secreted into the urine, is shown in Table 7. In the ETOP-OIL group, the etoposide concentration was approximately 1/2 to 1/13 of that in the Etop-sol group up to 6 h after administration and was undetectable by day 2. The concentration of drug in the kidney in the ETOP-OIL group was significantly lower at 30 min, 1 h, 3 h, 6 h and 1 day than in the Etop-sol group ($p < 0.01$–0.005).

In the urinary bladder (Table 8), the concentration of etoposide in the Etop-sol group was 18.6 μg/g at 15 min, increasing to 46.4 μg/g at 30 min and then decreasing thereafter. In the ETOP-OIL group, the concentration was lower than in the Etop-sol group at every time point. There were significant differences between the two groups at both 30 min and 1 h ($p < 0.05$ and 0.025).

Liver

In the Etop-sol group, the etoposide concentration in the liver, where the drug is metabolized, was more than two to three times that in the ETOP-OIL group over the first 3 h following administration (Table 9). Then the concentration in the Etop-sol group decreased less than in the ETOP-OIL group over the period from 6 to 24 h after administration. The concentration in the ETOP-OIL group was significantly lower at 15 min, 30 min and 1 h ($p < 0.025$–0.005), and significantly higher at 12 h than in the Etop-sol group ($p < 0.025$).

Table 7. Etoposide concentration in the kidney

Time after administration	Etoposide concentration (mean ± SE[a] μg/ml)		Statistical significance (*F*-ratio)
	ETOP-OIL	Etop-sol	
15 min	6.63 ± 2.24	11.67 ± 1.12	NS (4.02)
30 min	6.36 ± 1.28	22.84 ± 2.25	$p < 0.005$ (40.5)
1 h	4.37 ± 0.36	20.83 ± 1.06	$p < 0.005$ (214)
3 h	0.65 ± 0.04	8.26 ± 1.25	$p < 0.005$ (36.9)
6 h	1.21 ± 0.16	13.48 ± 1.48	$p < 0.005$ (67.5)
12 h	0.32 ± 0.02	0.45 ± 0.13	NS (1.15)
1 day	0.10 ± 0.03	0.29 ± 0.01	$p < 0.01$ (26.6)
2 days	ND	0.67 ± 0.12	—
3 days	ND	ND	—

[a]Mean ± SE for three experiments.
NS: not signficant.
ND: not detectable.

Table 9. Etoposide concentration in the liver

Time after administration	Etoposide concentration (mean ± SE[a] μg/ml)		Statistical significance (*F*-ratio)
	ETOP-OIL	Etop-sol	
15 min	4.49 ± 0.48	16.02 ± 0.99	$p < 0.005$ (109)
30 min	11.4 ± 1.7	37.1 ± 2.4	$p < 0.005$ (76.2)
1 h	6.43 ± 1.61	20.45 ± 3.03	$p < 0.025$ (16.7)
3 h	3.02 ± 0.69	7.30 ± 1.43	NS (7.24)
6 h	5.47 ± 1.13	2.07 ± 0.11	NS (8.88)
12 h	4.28 ± 0.74	1.35 ± 0.08	$p < 0.025$ (15.6)
1 day	1.39 ± 0.18	0.87 ± 0.19	NS (3.75)
2 days	1.79 ± 0.20	ND	—
4 days	ND	ND	—

[a]Mean ± SE for three experiments.
NS: not signficant.
ND: not detectable.

Discussion

The anticancer effects of etoposide depend not only on the concentration of the drug, but also on the duration of its actions.[8,9] In order to effectively control a peritoneal carcinomatosis with anticancer drugs such as etoposide, the drugs must be delivered to the intraperitoneal tissues at high concentrations for extended periods of time. Small water-soluble molecules such as etoposide in aqueous solution are, however, rapidly absorbed through blood capillaries located in the subperitoneum and are readily dispersed in circulating blood.[1] As such, it is difficult for aqueous dosage formats to maintain drugs at an efficacious concentration in the peritoneal cavity and its contiguous tissues for an extended period of time, when the drugs are administered intraperitoneally. Insoluble substances such as oil, however, are retained in the peritoneal cavity for a longer period of time and are absorbed gradually through lymph apparatus on the peritoneum.[1]

The present experiment demonstrates that etoposide concentrations in intraperitoneal tissues, such as the omentum and spleen, were much higher and remained high for a longer time in rats given ETOP-OIL than in rats given Etop-sol. This observation is in agreement with the superior therapeutic effects of ETOP-OIL noted in the treatment of peritoneal carcinomatosis in mice.[2] The etoposide concentration in blood plasma peaked at a relatively early time and decreased rapidly in rats given Etop-sol, whereas in rats given ETOP-OIL the concentration was maintained at a lower level for a longer time. In the extraperitoneal organs (lung, heart and bone marrow), the concentrations of etoposide in the Etop-sol group were higher than those in the ETOP-OIL group. These observations suggest that etoposide administered in the form of Etop-sol is more rapidly absorbed into the systemic circulation than etoposide administered in the form of ETOP-OIL, which is in agreement with the reduced systemic toxicity of ETOP-OIL noted in mice experiments.[2]

Conclusion

In conclusion, ETOP-OIL delivers greater concentrations of etoposide and is more selective in its delivery to the intraperitoneal tissues than Etop-sol.

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