Effective Treatment of Transmissible Venereal Tumors in Dogs with Vincristine and IL2

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Abstract. *Aim: To improve treatment of inoperable transmissible venereal tumors (TVTs) in dogs. Recently, we showed that TVT is sensitive to intratumoral treatment with interleukin-2 (IL2). In addition it is known that TVT is sensitive to intravenous treatment with vincristine. In the present study we tried to establish the therapeutic effect of intratumoral treatment with vincristine and IL2. Patients and Methods: We treated 12 dogs with TVT with 1-4 intratumoral treatments with vincristine and IL-2. Per treatment we used vincristine (0.5-0.7 mg/m^2) and IL2 (2×10^6 units). The injections were given at weekly intervals. Results: Early therapeutic effects were: three complete regressions, four partial regressions, three stable disease, and two progressive disease. Late therapeutic effects were established 45-60 months after the first presentation; there were five complete regressions, no partial regressions, nor stable or progressive diseases. Interestingly, all five dogs with late therapeutic effects were in good health. No tumor recurrence was noted. Conclusion: Intratumoral treatment of TVT with vincristine and IL2 appears to have impressive therapeutic effects.*

Transmissible venereal tumors (TVTs) frequently occur in dogs in tropical and sub-tropical countries. Many reviews have been written on this remarkable tumor syndrome (1-9). Briefly, the tumors are usually transmitted from one dog to another during mating when abraded mucosa is exposed to the tumor of an infected dog (1, 2, 8). Consequently the tumors grow mainly on the genitals. Initial lesions are superficial, pink to red, and 1-3 mm in diameter. Subsequently, multiple nodules fuse together forming larger, red, hemorrhagic, cauliflower-like, friable masses. The masses can be 5-7 cm in diameter and progress deeper into the mucosa as multilobular lesions with diameters that can exceed 10-15 cm (6). All TVTs have the same chromosomal aberration: whereas normal cells of dogs contain 78 chromosomes, cells from TVTs have 57-59 chromosomes (9, 10). Dogs with TVT are presented to veterinarian clinics where they have to be treated because untreated tumors grow and infect other dogs, and infected dogs make their home filthy due to bleeding and oozing serosanguinous fluid. The most frequent owner's complaint is hemorrhagic discharge (6).

There are several options for treatment. Firstly surgery; however, complete surgical excision cannot generally be achieved because of the location of these tumors on genitals (11). Consequently, after surgery most tumors recur. Secondly radiotherapy: TVT is sensitive to radiotherapy; however, radiotherapy is not available everywhere, such as in our clinics in Curacao. Thirdly chemotherapy; this is considered the treatment of choice for inoperable TVT. A variety of single-agent and combination multi-agent protocols employing cyclophosphamide, vinblastine, methotrexate and prednisolone are frequently used, but none has demonstrated superiority to employing intravenous chemotherapy with vincristine alone (9). Weekly intravenous administration of vincristine is presently the most effective and practical chemotherapy (6, 12, 13). The most frequent complication of vincristine therapy is the occurrence of local tissue lesions caused by extravasation of the drug during intravenous application, resulting in the development of necrotic lesions with crusts in non-tumorous tissue. Vincristine sulfate (0.5-0.7 mg/m^2), intravenously applied, once weekly for 3-6 weeks is usually effective (12). A fourth option is immunotherapy. Our research focuses on the treatment of cancer with local (intratumoral) application of interleukin-2 (IL2). Local therapy with IL2 is usually very effective; this in contrast to systemic IL2 therapy (14, 15).

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Local IL2 is therapeutically-effective against bovine ocular squamous cell carcinoma (16-18), canine mastocytoma (19), human nasopharyngeal carcinoma (20), and human bladder carcinoma (21). Recently we showed that TVT is also sensitive to local IL2 therapy (22). Apparently, at present, two therapeutic approaches are effective against inoperable TVT: intravenous chemotherapy with vincristine, and intratumoral immunotherapy with IL2. In preclinical studies (25) and in veterinary patients (26), we have shown that local IL2 and local chemotherapy may have synergistic therapeutic effects. Here we describe the therapeutic effect of combined local vincristine-IL2 (V/I) therapy. This study was performed in order to improve the treatment of TVT.

Materials and Methods

Dogs with TVT were included that were presented to the Veterinary Center Curacao, Ronde Klip, Curacao. Consecutive dogs with inoperable TVT were included. The diagnosis of TVT was pathologically confirmed in all animals by the presence of identical large, vacuolated tumor cells (Figure 1). TVT should be differentiated from mastocytoma, histiocytoma, and malignant lymphoma.

Table I shows characteristics of the dogs that were eligible for this study. Two dogs (no. 3 and 8) were not treated with V/I and one dog (no. 14) appeared to have another diagnosis. Therefore, 12 dogs with one tumor each were included. There were three males and nine females. The age ranged from 1-10 years; the median age was 3 years. There was one Rottweiler and the other dogs were of mixed breed. All dogs had natural (spontaneous) TVT. Natural TVT is contracted from other dogs and does not regress spontaneously in contrast to transplanted TVT that usually regresses spontaneously. Each patient was treated on an outpatient basis with the consent of the owner.

The tumors were treated with one to four weekly injections of V/I. The size of the tumors was measured at the first presentation; weekly just before injection of V/I; and two weeks after the last treatment. The smallest measured size of the tumor was used to calculate the therapeutic effect.

Vincristine and IL2 therapy. The tumors in the 12 dogs were treated with V/I intratumorally. These drugs were given one to four times, at intervals of one week. The size of the tumors was measured each week just before the tumors were treated with vincristine and IL2 as described above, and two weeks after the last drug injection. The dogs were treated by a veterinarian (M.H.).

IL2 (2×10^6 units; Novartis, Arnhem, The Netherlands) was suspended in 1.0 ml water. The dose of vincristine was 0.5-0.7 mg/m^2. We used Vincristine Sulfate Injection, USP, a sterile, preservative-free, single-use only solution. Each milliliter contained 1 mg vincristine sulfate. Vincristine and IL2 were given intratumorally, in two separate injections of 1 ml each, as intratumoral application of drugs is usually more effective than systemic application of a drug (14). If the tumor was too small to inject about 2 ml drug-containing fluid, then the fluid was also injected peritumorally.

Tumor sizes were measured with callipers as length, width and height immediately before the V/I injection. The product of these measurements (*i.e.* the volume) at first presentation was compared to the volume of the treated tumors. Therapeutic effects were described as complete regression (CR: tumor size 0% of the original tumor), partial regression (PR: tumor size of 1-50% of the original tumor), stable disease (SD: tumor size of 50-150% of the original tumor), and progressive disease (PD: tumor size of over 150% of the original tumor).

Table I. *Characteristics of the dogs included in this study.*

Number of dog	Breed	Gender	Age (years)
1	Mixed breed	M	10
2	Mixed breed	F	1
3 a			
4	Mixed breed	M	3-4
5	Mixed breed	F	x
6	Mixed breed	F	x
7	Mixed breed	M	3
8 a			
9	Mixed breed	F	5
10	Mixed breed	F	ca 6
11	Mixed breed	F	8
12	Rottweiler	F	2
13	Mixed breed	F	x
14 b			
15	Mixed breed	F	1

a, No treatment; b, no tumor; M, Male; F, Female; x, age unknown.

Histology. We wondered by which mechanism tumor regression was induced. Therefore hematoxylin and eosin-stained sections of the tumors were prepared for microscopic diagnosis and to study the changes induced by the therapy.

Results

The effect of intratumoral V/I therapy on the size of the tumors. Twelve dogs with a TVT were treated. The tumors were treated with 1-4 weekly injections of V/I. The size of the tumors was measured at first presentation, and weekly just before injection of the drugs, and two weeks after the last treatment. The smallest measured size of the tumor was used to calculate the therapeutic effect. The effect of weekly treatments with V/I in and around the tumors is shown in Table II. There were three CRs, four PRs, three SDs, and two PDs.

We wondered whether therapeutic effects were long lasting. Therefore we asked the owners of the dogs whether their dog was still alive, had a tumor, was in good health, and whether the tumors had recurred. This was 45-60 months after the first presentation. Median age at first presentation was 3 years; the median lifetime of large dogs is about 10 years. To our surprise, five dogs were still alive, all five were in good health, and there had been no tumor recurrence. In four out of five cases, the tumor had not regressed

Table II. *Early therapeutic effect of treatment with Vincristine and IL-2*

Number of dog	Tumor size at first presentation (cm^3)	Number of treatments	Tumor size (cm^3) depending of number of treatments	Smallest measured tumor size (cm^3)	Smallest size (% of size before treatment)	Therapeutic effect
1	14.4	0	14.4		0.0	CR
		1	3.9			
		2	0.9			
		4	0	0.0		
2	1	0	1	3.9	3.9	PD
		1				
3 a						
4	9	0	9	0.0	0.0	CR
		1	1.6			
		2	0.0			
5	55	0	55	4.9	8.9	PR
		1	7.7			
		2	6.1			
		3	4.9			
6	2.8	0	2.8	6.7	2.4	PD
		1	23.9			
		2	6.7			
		3	26.4			
7	11.6	0	11.6			PR
		1	5.8	5.8	50	
8 a						
9	18.9	0	18.9		95.8	SD
		1	18.1	18.1		
		2	26.9			
10	0.4	0	0.4	0.6	150	SD
		1	0.6			
11	19.3	0	19.3			
		1	17.8			
		2	14.8			
		3	0.8	0.8	3.9	PR
12	19.3	0	19.3			
		1	30.5			
		2	23.4			
		3	10.9	10.9	56	SD
13	34.5	0	34.5			
		1	18.9			
		2	23.0			
		3	7.7	7.7	3.3	PR
14 b		0				
15	1.0	0	1.0			
		1	0.3			
		2	0.1			
		3	0.0	0.0	0.0	CR

a, No treatment; b, no tumor.

completely when the early effects were measured. This shows that tumor regression induced by V/I is a slow process (Table III).

Histopathological effects of tumor regression induced by V/I therapy. Figure 1 shows that TVT cells of untreated tumors are large uniform cells growing in solid fields. The cells have large nuclei with coarse chromatin and distinct nucleoli. Unlike most malignancies, TVT cells exhibit little polymorphism, as might be expected from the cell-line nature of this tumor. The tumor consists mainly of tumor cells.

Figure 2 shows an influx of leukocytes after treatment of a tumor with V/I. The tumor cells are large and lightly stained. The tumor had been invaded by the smaller, more

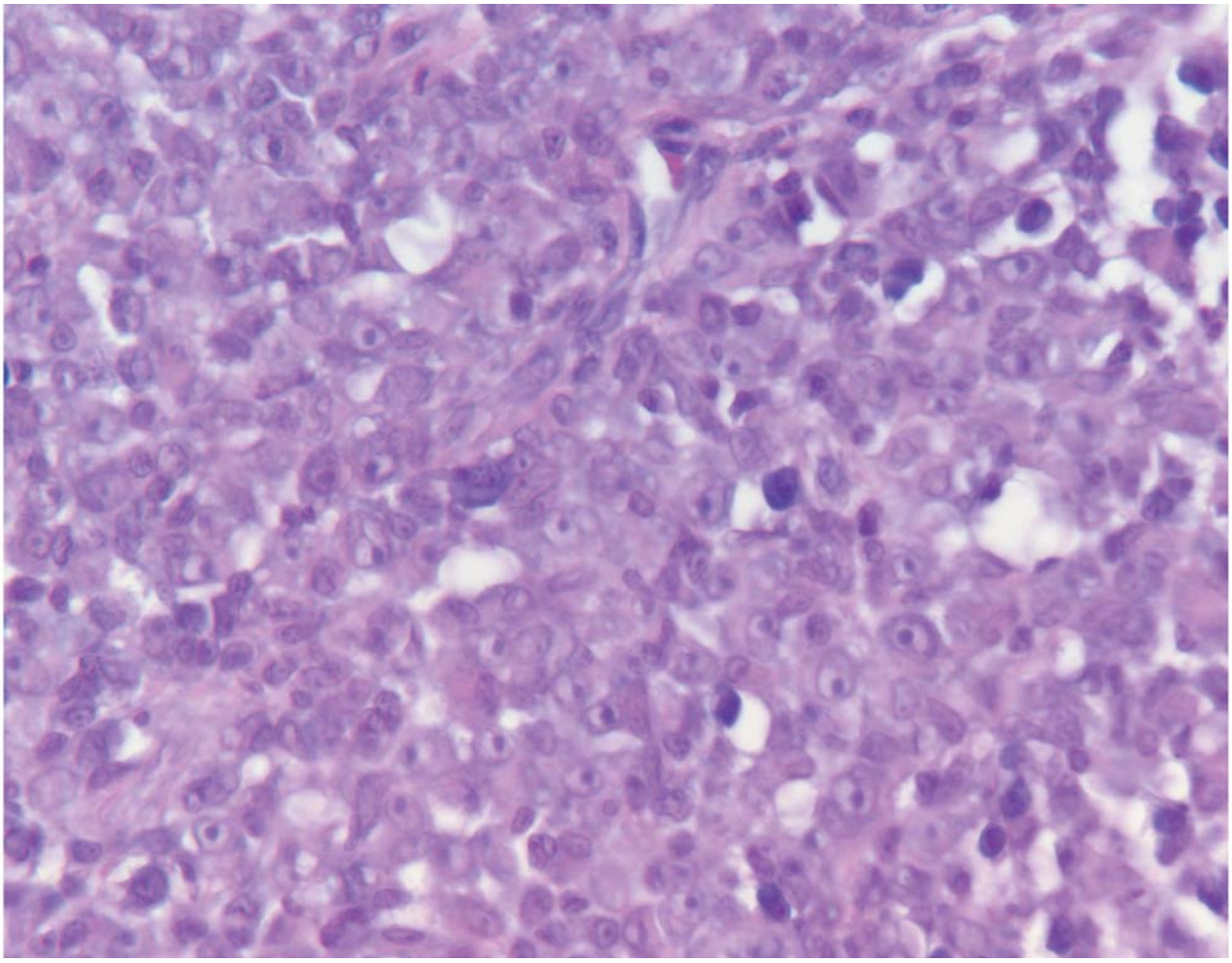

Figure 1. *Hematoxylin and eosin-stained section of an untreated transmissible venereal tumor showing the large vacuolated tumor cells. Magnification: objective ×40.*

darkly-stained lymphocytes. The scattered large cells with large vacuoles are starry-sky macrophages. The magnitude of the influx of inflammatory cells is very variable in different tumors.

Figure 3 shows complete tumor regression after treatment with V/I. Tumor cells had been replaced by reactive fibrotic tissue and inflammatory cells. Interestingly, a macroscopical, measurable tumor persisted, but this tumor did not contain malignant cells. This implies that the tumors like the one shown in Figure 3 are histologically CRs, whereas they are PRs according to the macroscopical size of the tumor. This means that the number of complete cures is underestimated when the macroscopic technique is used to establish the therapeutic effect. This occurred in dog 11. We, apparently, underestimated the therapeutic effect with the macroscopical scoring technique in this dog.

Table III. *Late therapeutic effects*

Number of dog	Therapeutic effect 3 months after last treatment	Time interval (months) between first presentation and writing of manuscript	Late therapeutic effect
1	CR	60	Lost
2	SD	60	CR
4	CR	50	Lost/moved abroad
5	PR	51	Dead/due to tumor?
6	PD	58	CR
7	PR	58	Dead/car accident
9	SD	52	CR
10	SD	52	Lost
11	PR	47	CR
12	SD	47	Dead/aged dog
13	PR	47	Lost
15	CR	45	CR

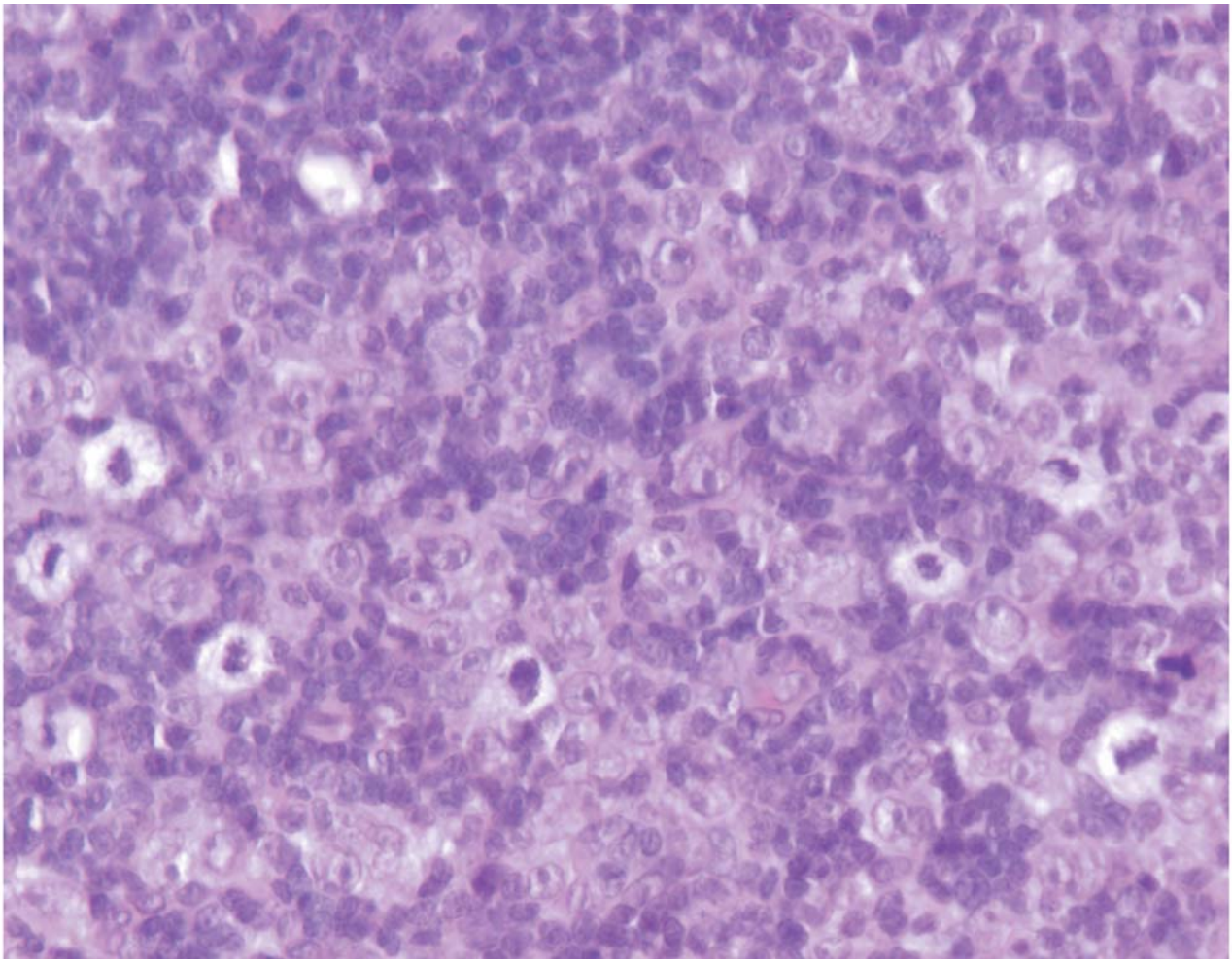

Figure 2. *Influx of inflammatory cells after treatment with vincristine and interleukin-2. Tumor cells are large and light, whereas inflammatory cells are smaller and darker. Compare this histology with that shown in Figure 1. Hematoxylin and eosin-stained section. Magnification: objective ×40.*

Toxic side-effects. Intratumoral injection of V/I caused nausea for a day. In addition, toxic side-effects of vincristine were observed if vincristine was spilt outside the tumor, resulting in local tissue necrosis. After some time, however, only a small scar was visible.

Costs. Both vincristine and IL2 cost about 200 € each per complete treatment of a dog. Therefore, to treat a dog with V/I costs about 400 €.

Discussion

We showed that intratumoral V/I injection resulted in three CRs, four PRs, three SDs, and two PDs. Thus in seven out of the 12 (58%) animals there was a worthwhile therapeutic effect (CR or PR) of V/I. These results may be an improvement compared with the results obtained with treatment of TVT with intratumoral injection of IL2 only (22). The results of IL2 treatment alone in eight dogs were one CR, two PRs, four SDs, and one PD. This is a therapeutic effect (CR and PR) in three out of eight (37% of the cases) (23). The Chi-square test shows a trend for a significant difference between therapeutic effects after V/I compared with IL2 alone. We calculated that a significant effect might be obtained if 30-40 dogs were included in each group.

The choice of the therapy of TVT also depends on other factors such as the frequency of treatments: IL2 requires one treatment that needs only one visit to the clinic (24), whereas vincristine has to be applied 3-6 times (12) requiring 3-6 visits. Furthermore, vincristine that is applied to free roaming dogs may come in contact with small children and pregnant women. This is undesirable, as vincristine is very toxic; whereas IL2 is not toxic.

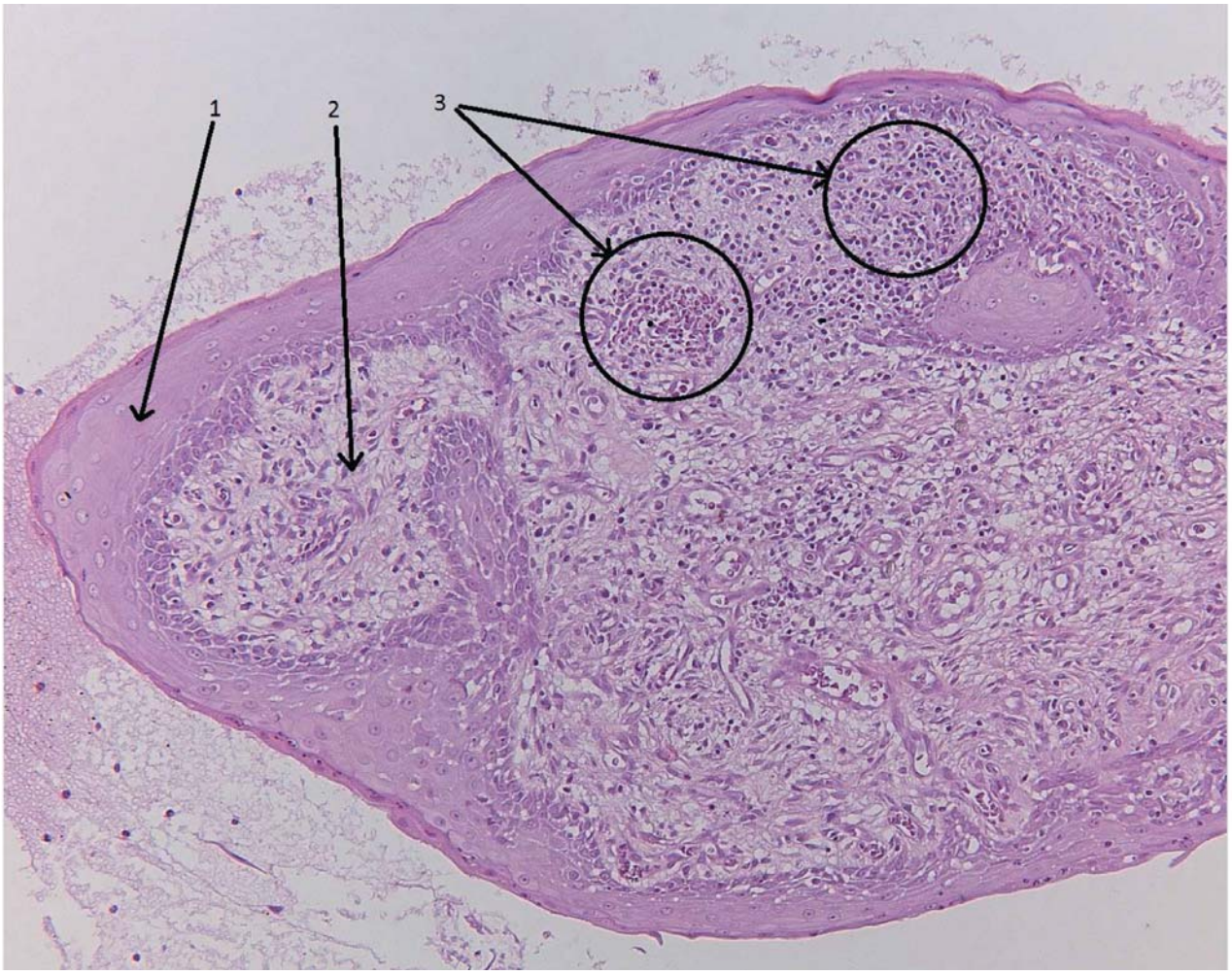

Figure 3. *Case of complete tumor regression. Tumor cells have been replaced by connective tissue. 1. Epithelium; 2. Connective tissue; 3. Influx of lymphocytes at the top of the section. Hematoxylin and eosin –stained section. Magnification: objective ×10.*

We studied the histopathology of TVT rejection with the aim of gaining more insight into tumor regression induced by V/I. It is important to note that all TVT tumor cells exhibited the same morphology (Figure 1). At one week after injection of V/I, histology showed invasion of lymphocytes (Figure 2). In addition, there were small numbers of macrophages, granulocytes, and apoptotic tumor cells. At the end of the process, tumor cells had been replaced by reactive connective tissue cells with hardly any or no tumor cells (Figure 3). This is understandable as healing of dead tissue (*e.g.* due to heart infarction) is a time-consuming process. In the present study, the observation that tumor tissue after some weeks is replaced by connective tissue is compatible with such a time consuming process (25, 26).

Remarkably, in the 12 tumors that we studied, never were there fields of necrotic cells found in the tumor. Macrophages and granulocytes were also rare. This might be a result of the two-week interval between the last V/I injection and the histological examination. From studies of heart infarction, it is well-known that the presence of granulocytes is limited to the first two days after the event, and necrotic tissue is rapidly replaced by young connective tissue, usually within a week after infarction (27). A similar process may occur in TVTs treated with V/I. In the present study, we saw only rather late processes after V/I injection.

Conclusion

Local application of V/I to treat TVT gives long-lasting good therapeutic effects, namely cure without tumor recurrence. Treatment with V/I can kill virtually all tumor cells of a TVT. After successful treatment with V/I, the tumor tissue may be replaced by connective tissue.

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