Intratumoural Interleukin-2 Therapy Can Induce Regression of Non-resectable Mastocytoma in Dogs

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Abstract. *Aim: Mast cell tumours (MCT) are common skin tumours in dogs. If complete surgical removal of the tumours is not possible, then another therapy is needed. In the current study we tested the therapeutic effect of intratumoural injection of interleukin-2 (IL-2). Materials and Methods: Seven dogs had non-resectable cutaneous MCT. The tumours were injected with 4.5×10^6 IU IL-2. Results: The early clinical effects in the seven dogs with cutaneous MCT were: complete regression (CR) in two dogs; partial regression (PR) in four, and stable disease (SD) in one dog. The final clinical effects were CR in three dogs, PR in two dogs, and PD in two dogs. Conclusion: This pilot study shows that intratumoural IL-2 application can exert an anti-MCT effect. A larger study would be required to precisely establish the magnitude of the therapeutic effect against MCT. A single application of IL-2 in cases of non-resectable MCT has no observable side-effects.*

Mastocytomas (MCT) are common tumours of the skin in dogs (1). MCTs represent 6% of all tumours in the dog and 13% of all skin tumours in the dog (2). The mean age of dogs with cutaneous MCT is approximately 9 years. The biological behaviour of MCTs is variable; some dogs have solitary tumours, while others have multiple tumours (3). Surgical removal of MCTs is the treatment of choice. Wide margins (2 to 3 cm) are required because of the tendency of the tumour cells to spread around the tumour (4, 5). If complete removal with margins is not possible due to the size or location, additional treatment such as radiotherapy or chemotherapy may be necessary (6, 7).

IL-2 is a cytokine that has antitumour effects when given systemically to human patients (8, 9, 10). The disadvantage of systemic IL-2 therapy is that the therapeutic effect is limited to about 20% objective responses in patients with metastasized renal cell carcinoma and metastasized melanoma (9). In addition, systemically-applied IL-2 causes severe side effects due to the generalized vascular leakage syndrome (9, 11, 12).

Local IL-2 application (intratumoural, peritumoural, intravesical) is far more effective than systemic IL-2 therapy. Locally-applied IL-2 causes hardly any side-effects (13-18). Our group is specialised in cancer treatment by local application of IL-2 (16). We have obtained good therapeutic effects against cancer in veterinary patients such as bovine ocular squamous cell carcinoma (19-21), bovine vulval papilloma and carcinoma complex (22, 23) and sarcoids in horses (24). In human patients, we have shown that local IL-2 application is therapeutically-effective against superficial bladder carcinoma (25) and nasopharyngeal carcinoma (15). The groups of Radny (14) and Pfohler (26) showed the effect of local IL-2 application on melanoma. IL-2 is also effective in a variety of other cancer types (10, 16).

Local injection of IL-2 leads to extravasation of fluid and erythrocytes at the injection site (11, 12, 27). This causes stagnation of the blood flow, leading to tumour cell death. At a later stage, leucocytes migrate to the dead tumour cells; this stimulates the immune response (16, 28, 29). In this article we describe a pilot study on the therapeutic effects of intratumoural application of IL-2 in dogs with non-resectable MCT.

Materials and Methods

Dogs with cutaneous MCTs were presented at our clinics in Oisterwijk and in Gouda. Inclusion criteria for this study were: (a) Cytologically-confirmed MCT that was thought to be non-resectable; tumours were regarded to be non-resectable when surgery required

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Key Words: Mastocytoma, interleukin-2, local therapy, immunotherapy, dogs.

Table I. *Dogs with cytologically-proven mast cell tumours.*

No	Breed	Gender	Age (years)	Size (mm)	Metastases
D1	Mixed breed	F	11	65×35×2	N
D2	Bernese Mountain dog	M	7	32×38×32	N
D3	Yorkshire terrier	F	14	22×30	N
D4	Jack russell terrier	F	7	20×12	N
D5	Rottweiler	MN	5	12×16	N
D6	Bouvier des flandres	F	6	10×10	N
D7	Golden Retriever	M	11.5	28×28	N
M1	Boxer	F	7	60×60	Y
M2	Labrador retriever	FN	10.5	50×50×20	Y
M3	Bernese Mountain dog	M	3.5	35×35	Y

No, Patient number; D, dog with skin tumour; M, dog with metastasis; F, female; M, male; N, neutered; Initial tumour size in mm in 2 or 3 dimensions (it is not always possible to measure tumour sizes in 3 dimensions). N, no; Y, yes.

Table II. *Location of the skin tumours*

No	Location
D1	Distal left fore limb
D2	Large foot pad of right fore limb
D3	Caudal knee of left hind limb
D4	Planum nasale
D5	Lateral of the preputium
D6	Planum nasale
D7	Large foot pad of right fore limb
M1	Distal right hind leg
M2	Ventral vulva and caudal skin abdomen
M3	Distal left hind limb

See Table I for dog identity.

Table III. *Therapeutic results of intratumoural IL-2 therapy.*

	Early clinical effect[a]			Final clinical effect[b]		
No	From[c]	To[d]	Effect	At[e]	Effect	TRD
D1	1	24	PR	29	PR	N
D2	7	7	PR	8	PR	TOP
D3	7	10	PR	10	CR	N
D4	1	2	SD	5	PD	Y
D5	3	21	CR	21	CR	N
D6	4	5	CR	6	PD	TRD
D7	2	4	PR	16	CR	N
M1	7	10	SD	18	PD	Y
M2	1	1	SD	2	PD	Y
M3	1	5	PD	35	PD	Y

[a]Early change in tumour growth after local IL-2 therapy; [b]Clinical effect at last visit; [c]Time point in months after therapy that the early effect is visible; [d]Last time point that the early effect was observed; [e]Time point of final clinical effect; CR, complete regression (no tumour visible): PR, partial regression (tumour size <50% of the original tumour): SD, stable disease (tumour size 50-150% of the original tumour): PD, progressive disease (tumour size >150% of the original tumour): TRD, tumour-related death; TOP, taken of protocol; N, no; Y, yes.

amputation that was not acceptable to the owner. Animals with metastasis were accepted. (b) Clinical performance 0 (no clinical signs) to 3 (some tiredness and dyspnea). Exclusion criteria were: (a) Dogs that were thought to have surgically-resectable MCT. (b) The presence of other types of tumor. (c) Clinical performance 4 or 5 (poor condition: not able to care for themselves or close to death). (d) Previous treatments. All patients presented to our clinics fulfilling these criteria were included. The WHO or Zubrod clinical performance scale was used (30). Staging of the MCT was not performed as the group of 10 dogs was regarded to be too small for statistical analysis of subgroups. Urine analysis was not performed. H1/H2 antagonists were not needed and were not used. We performed an abdominal ultrasound search for metastases, especially in the liver and the spleen. Metastasis was confirmed cytologically.

Tumours in 10 dogs were unresectable and these were treated with intratumoural IL-2 therapy only. Three of these dogs had metastases: two of them had metastasis in the regional lymph node and one had a local metastasis (new tumour around the primary tumour) in the skin. The animals received one injection of 4.5×10^6 units of human recombinant IL-2 (Proleukin®, Novartis, Arnhem) in 0.25 ml solution in the primary tumour. This dose and the volume of the suspension were based on results described by Den Otter *et al.* (16). Only one injection of IL-2 was given, as experience has shown that one dose is usually effective (16). An exception was described by Kusnierczyk and others (31), but in their study in mice, additional IL-2 injections were given when the tumour started to re-grow; of course we could not do this for logistic reasons. Patients with a primary skin tumour and metastasis were included because in several studies it was shown that treatment of the primary tumour with IL-2 could lead to the regression of a metastasis (13, 29, 32).

Dogs were examined every month. Special attention was paid to the tumour size and the regional lymph nodes. Therapeutic effects are expressed as complete regression (CR): no tumour visible, partial regression (PR): tumour size less than 50% of the original tumour), stable disease (SD): tumour size 50-150% of the original tumour, and progressive disease (PD): tumour size more than 150% of the original tumour or tumour growth leading to death. Dogs were euthanized when they were too ill. We distinguished early and final clinical effects. Early effects are effects measured during the first seven months after treatment. Final clinical effects are effects at the last visit of the living animal (before death due to cancer or other causes, or due to exclusion from further analysis).

Ethics. This research was performed in compliance with the guidelines of the Department of Clinical Sciences of Companion Animals, Utrecht. Written informed consent to treat the animals was given by the owners of the dogs.

Results

Ten dogs with non-resectable MCTs were treated with IL-2 injections into the primary tumour. Tables I and II present information about these dogs and their tumours. The age of

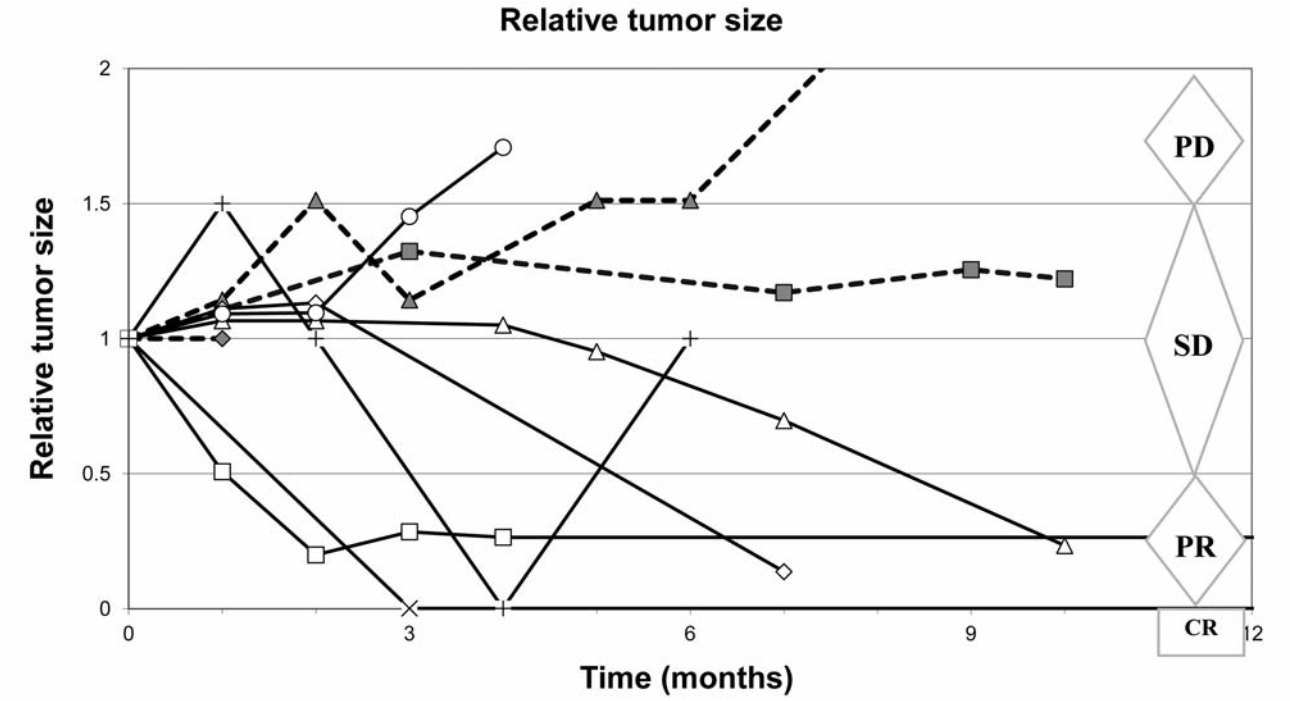

Figure 1. *Growth kinetics of skin tumours after local IL-2 therapy-only. Closed symbols are data from animals with metastases. Open symbols are data from animals without metastases; Six out of seven cases of primary tumours without metastases regressed. Skin tumours with metastases tended to grow. Tumour regression is often a slow process, requiring more than 6 months.*

the dogs was 3.5 to 14 years, with a mean of 8.8 years. The longest diameter of the tumours ranged from 10 to 65 mm (median=32 mm).

We did not observe any side-effects of local IL-2 treatment of MCT; thus no supplementary medication was required.

Non-metastasized tumours. Seven dogs had locally advanced tumours only (D1–D7; Tables I and II). Therapeutic effects are shown in Table III and Figure 1. Local treatment of mastocytomas D1–D7 led to clear early clinical effects: 2 CRs, 4 PRs, and 1 SD. The CRs lasted for 21 months (D5) and 6 months (D6). The final clinical effect observed at 5-29 months after treatment was 3 CRs, 2 PRs, and 2 PD. Consequently, in 5/7 animals there was a worthwhile clinical effect. Figure 1 shows that tumour regression induced by intratumoural IL-2 may be a slow process; regression of 4/5 tumours required more than 6 months. In one of the dogs with a PR (D2), the tumour became surgically removable. Therefore this dog was excluded from further analysis.

Metastasized tumours. Dog M1 had a metastasis in the regional lymph node. The primary tumour showed SD from 7 months to 10 months after treatment. Thereafter tumour growth progressed leading to death of the dog.

Dog M2 had a skin metastasis near the primary tumour. This dog died 2 months after treatment due to the tumour, without noticeable change of the size of the primary tumour, but the metastasis was growing.

Dog M3 had a metastasis in the regional lymph node. The primary tumour did not change in size after IL-2 treatment of the primary tumour up to 5 months after treatment. Only after 35 months' tumour progression did this dog die from MCT.

In dogs M1–M3 their metastases did not regress after IL-2 application to the primary tumour.

Figure 1 shows the different therapeutic effects in animals with non-metastasised skin tumours and those with primary tumours with a metastasis: those with non-metastasised tumours usually showed a therapeutic effect (CR, PR, SD), whereas the primary tumours with a metastasis did not regress.

Discussion

In this article we describe local (intratumoural) IL-2 application for non-resectable MCT in dogs. The therapeutic effect against inoperable, non-metastasized MCT is convincing: two dogs (numbers D5 and D6) had a CR. In D5, this lasted for at least 21 months. Three dogs (D1–D3) had a PR with a 4- to 7-fold decrease in tumour size. The

tumour in dog 2 regressed sufficiently for surgery to be undertaken. A longer follow-up might have resulted in even better results, as the tumour response to IL-2 is often slow. Such a slow regression has also been reported in cattle with ocular squamous cell carcinoma treated with local IL-2 therapy (19-21). Slow tumour regression is also the case in MCT, as illustrated in Figure 1, where the regression of tumours of four dogs required more than six months.

In the three dogs with metastasized MCTs no therapeutic effects were obtained, as early effects comprised of two SDs and one PD, and the final clinical effects were three PDs. The lack of effect on the non-treated metastases was rather surprising as in other models there were therapeutic effects on non-treated tumours at a distant site. This occurred with transplanted SL2 lymphoma in mice (13, 29, 32), transplanted carcinoma in the ears of rabbits (33) and bovine vulval papilloma and carcinoma complex (22).

We explain the mechanism of local IL-2 activity as follows: IL-2 induces vascular leakage leading to oedema. This inhibits the circulation of fluid, causing lack of oxygen and nutrients for the tumour cells. Many of these tumour cells die. The tumour debris is phagocytosed and this leads to immunity (16). This immune process and in particular the phagocytic process, requires time. This explains why tumour regression requires months.

Local IL-2 treatment of MCT in dogs is easy. We did not observe any side-effect of local IL-2 treatment of MCT (erythema, necrosis, pain, gastrointestinal effects, *etc*.). Den Otter *et al*. (16) reviewed the toxic effects of local IL-2 application to human patients with cancer. There were 24 articles with an abstract mentioning toxic side-effects. In 20, there were no or only minor side effects reported, one reported some side-effects (1/9 patients had fever grade I and 7/9 patients had hypotension grade I-II) and 3 mentioned more serious side-effects. The latter three studies used very high doses for intrapleural, intraperitoneal or subcutaneous administration. Hence, side effects are mainly expected after very high doses of IL-2, but not after the relatively low doses that we applied locally. Such massive doses act in a pseudo-systemic manner. Much of the IL-2 is absorbed and as a consequence will give generalised effects similar to systemic treatment.

Usually it is not necessary to sedate the animal. Only a single treatment is sufficient in most cases (16, 33). A clear exception was the treatment of transplanted MC38 colon carcinoma in mice, where multiple IL-2 treatments led to better therapeutical effects than did a single treatment (31).

The results described here warrant further studies on local IL-2 application for MCT in dogs. These studies should comprise of more animals than we included, compare therapeutic effects after single and multiple local IL-2 applications, and should use stratification to allow for comparison of the therapeutic effects on MCTs with different grades of malignancy. The effects on non-treated metastases also requires further study.

Conclusion

A single application of IL-2 for non-resectable MCT is feasible, has no observable side- effects, does not require invasive surgery, does not need frequent irradiation sessions with sedation, does not need multiple toxic chemotherapy, and is cheap. This study shows convincingly that local IL-2 application can exert a therapeutic effect against MCT. The results warrant a larger study with more patients to establish the anti-MCT effect more precisely.

Conflicts of Interest

None of the Authors of this article has financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the article.

Acknowledgements

We acknowledge receiving a grant of the Stichting Nationaal Fonds tegen Kanker (SNFK), Amsterdam, to JJLJ

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Received October 4, 2012
Revised November 23, 2012
Accepted November 26, 2012