



*Mini-review*

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## EFFECT OF ANAESTHESIA ON TUMOUR METASTASES

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### SUMMARY

Malignant tumour diseases are important in our study on pathology of small animals. Metastases arise primarily from non-benign tumours and this situation could provide bases for difficult control of the disease, with the attendant high mortality. Surgery is often one of the invasive means of controlling complications of cancer and its metastases. That then makes it imperative to know how this and its accompanying anaesthesia could affect the generation of metastasis in such patients. The present study reviews the available data regarding effects of anaesthesia on tumour metastasis. It starts by giving some major highlights on the mechanism of the metastatic process and antitumour immunity. Consequently it addresses the questions about the effects of different anaesthetic agents on the NK cells count and their activities and some other factors connected with the metastatic process.

**Key words:** tumour, metastasis, anaesthesia, NK cells, dog, cat

### EPIDEMIOLOGY OF TUMOURS IN SMALL ANIMALS

Cancer is a genetic disease affecting either humans or animals. Dogs suffer from cutaneous cancer thirty-five times more frequently than humans, from breast cancer four times more than humans, from bone cancer eight times more than humans and from leukaemia two times more than humans (Cullen et al., 2002). The only tumour types, which are encountered more often in humans than in animals, are lung cancer and gastrointestinal cancer. The reasons are due to tobacco smoking in humans, a potent cancer-inducer, and species susceptibility to tumour growth of the stomach and intestines

With regard to the breed predisposition to tumour growth, among dogs, it was found that the most affected were the Boxer, Golden Retriever, Rottweiler, which demonstrated a relatively early appearance (Cullen et al., 2002). Breeds with lower frequencies of tumour were the Boston terrier, English bulldog, and Cocker Spaniel, while the least susceptible were the beagles, poodles, collies, Great Danes. The reason for this breed

predisposition is unknown. However, it is thought that those with higher frequencies could have inherited some abnormal genes, a condition that is known to give rise to neoplastic cell transformation (Sorenmo, 2003). Cancer is not a problem for thoroughbred animals only. Mixed breeds equally stand the risk of cancers as thoroughbred animals. However, breed characteristics define the tendency to develop a certain type of tumours. For example, giant breeds such as the Saint Bernard and Great Dane often develop osteosarcoma than those of the medium size (Chun & de Lorimier, 2003). Dogs and cats with white fur and skins are susceptible to cutaneous carcinoma under the impact of ultra-violet radiation. On the other hand, black and dark coloured animals frequently suffer from melanoma (Rosenthal, 1998).

Intact female dogs have been shown to exhibit high risk of developing breast cancer 7 times more often than early castrated females of up to 2 years of age (Sorenmo, 2003). Oestrogens and progesterone are hormones with potent stimulator effect on mammary gland tumours in animals and humans (Key & Pike, 1982).

Incidences of tumour diseases in cats are roughly half of those in dogs. For example, the frequency in cats is 158-470/100,000 whereas in dogs it is 381-

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1126/100,000 (Cullen et al., 2002). In return for it, the malignant incidences of tumours in cats (over 70 % of all tumours) are more than they are in dogs (over 35% of all tumours). Specifically, haematopoietic, cutaneous and soft tissue tumours are prevalent in cats, compared to other tumour types.

Cell biology has helped in the description of these tumours and results from it have shown many different histological types with varied anatomical localizations. For example, about 35-45% of all feline tumours have been found to consist of cutaneous and soft tissue tumours while haematopoietic neoplasm comprised the remaining 30-40% (Hardy, 1981). Generally, mammary gland tumours are common in the intact females, compared to the castrated females and males. However, the relationship between gender and tumour development in cats has not been established (Gabor et al., 2000). With regard to age of onset, most tumours affect cats over 5 years of age. However, some tumours, such as lymphosarcoma, present themselves in a bimodal age mode. Just like in the dog, cats show breed predisposition to certain tumour types. For example, Siamese cats are frequently afflicted by intestinal adenocarcinoma but rarely by cutaneous squamous cell carcinoma.

In a retrospective study of small animal tumour epidemiology in Bulgaria mammary gland neoplasm, with over 60% malignancy, was found to be prevalent in dogs, especially among the Bolognese breed aged over 8 years (Dinev et al., 2002). Feline neoplasm epidemiology was not investigated.

Based on the above data and others obtained from many studies knowledge about tumour biology, especially as it affects humans, is on the increase.

### **MECHANISM OF TUMOUR METASTASIS AND ANTITUMOUR IMMUNITY**

The ability to invade and metastasise is a unique feature of malignant neoplasm. The disease caused by the metastasis, unlike the primary tumour, underlies the difficulty in treatment and the high mortality rate. The process of metastasis generation is complex and multistage (Cullen et al., 2002). It begins with vascularisation of the primary tumour followed by usurpation of the affected tissue, release of tumour cells from the primary affected area, distribution of tumour cells to the distant sites, blocking the microcirculation

of organs, going out of tumour cells of blood and lymphatic vessels, infiltration and proliferation in the new-captivated part of the body.

In order to become invasive the tumour must perforate basal membrane of endothelial cells. Because of that, the lymphogenic distribution of tumour cells predominates over haematogenic, since the lymph vessels do not have a basal membrane. Tumour cells can spread out by lymph, by blood, and by visceral surface adhesion. Ultimately, either they get into the *v. cava cranialis* through the thoracic duct or into *v. portae*, as they stop in the first capillary bed which they gain. Mammary gland tumours, neoplasm of the skin, soft tissue, bones, and thyroid glands get to the lung but gastrointestinal or pancreatic tumours get to the liver. The tendency of definite organ invasion is typical for some tumour cells which bind to tissue-specific endothelial markers or possess receptors to haemokines, such as melanoma (with preferably brain localisation of metastases) and prostate gland carcinoma (with metastasis localised in the bones).

It is found that very small number of the tumour cells (0.01% - Postre & Fidler, 1980) entering in the circulation survives and settles after some metastases. Most of them die through circulator turbulence or through conflict with the immune cells. The longer they stay in circulation the lower is their chance to metastasise. Regional lymph nodes are extremely important in the fight against tumour cells spread. The cell-mediated immunity is predominantly engaged in this process and the role of humoral immunity is not entirely clear (Rosenthal, 1998). Tumour cells may stimulate cytotoxic T-lymphocytes but tumour progression is associated with amplification of the T-suppressor activity in the regional lymph nodes with the beginning of metastasis. The importance of the immune system for tumour development is proven by the fact that the cases of cancer in immunodeficiency conditions are very high. For instance, individuals with congenital immunodeficiency experience 2-4% higher frequency of cancer, and over half of these are identified as non-Hodgkin's lymphoma (Rosenthal, 1998). The same situation occurs in patients with different organ transplantation who are on immunosuppressive drugs (Wooldridge, 2002). On the other hand, the critical role of the immune system in the pathogenicity of some oncogenic viruses is well known (retroviruses, herpes viruses, poxviruses, adenoviruses). Malignant

transformation of the cells is associated with phenotype membrane changes, such as loss of normal antigenic constituents or induction of neo-antigens provoking an immune response. The immune response may be induced by the expression on tumour cells strong tumour-specific antigens which are recognised by macrophages. Activated macrophages start producing cytokines, mostly interleukin-1 (IL-1) and tumour necrosis factor (TNF), thus giving signals to other immune cells (T-lymphocyte precursors) that it is time to differentiate to T-helper cells classes 1 (Th1) and 2 (Th2), T-suppressor cells (Ts), cytotoxic T-lymphocytes (Tc), depending on the type of cytokine produced. Beside this primary signal of antigen presentation to cytotoxic T-Ly, macrophages have a direct cytotoxic effect on tumour cells by their liposome enzymes and free oxygen radicals. Since antigens are located on the surface of tumour cells, (which in fact are malignant transformed proper cells) a cell-mediated cytotoxicity is induced. The main agents in cell-mediated cytotoxicity are Tc, K cells and NK cells. Tc attack target cells only if the former recognise antigens on the surface of antigen-presenting cells and their principal role is to eliminate virus-infected cells. K cells are members of monocyte-macrophageal system and are associated with antibody-dependent cytotoxicity during which their targets are cells bound to antibodies. K cells bind to the Fc-fragment of IgG whereupon they kill antibody-connected cell. Some cells are able to destroy tumour cells without an initial recognition of specific tumour antigens. This type of effectors cells are the NK cells. They represent 2-5% of peripheral lymphocytes. Their role in antitumour immunity is determined by their ability to recognise determinants located on the surface of tumour cells. The ability of the NK cells to recognise and destroy their targets depends on the grade of differentiation of these targets. Therefore, the killing potential of NK cells correlates with the rate of malignancy (Rosenthal, 1998).

#### **THE POTENTIAL ROLE OF ANAESTHESIA IN THE GENERATION OF METASTASIS**

One of the complex therapies in cancer is surgery, that is, surgical excision of the cancerous tissue. Surgery is necessary also for arresting some complications due to tumour progression, such as hollow organ obstruction, unset bone fragments and nerve roots compression. Unfortunately, the disease itself,

as well as some factors of perioperative period, results in immunosuppression, thus creating preconditions for tumour to metastasise. In cancer either the humoral or the cell-mediated immune response is suppressed. Factors resulting in immunosuppression in perioperative period are some anaesthetic agents, opioids, surgery, stress (both operative and anaesthesiological), temperature changes, blood transfusion and pain (Vallejo et al., 2003). The principal immunological factor connected with the spread of tumour cells in the body is the activity of natural killer cells-NK cells (Ben-Eliyahu & Page, 1992). The decreased activity of NK cells during the perioperative period is associated with the enhanced risk of mortality and recurrence of tumour growth in cancer of different organs (Pross & Lotzova, 1993). The activity of these cells is suppressed either by surgery or by anaesthesia. In some recent *in vivo* studies it was found that operative stress without anaesthesia resulted in 3-4-fold increase in the spread of tumour metastasis in comparison to anaesthesia only (Bar Yosef et al., 2001; Page et al., 2001). However, the effects of anaesthesia may contribute to either increase or decrease of this negative influence of surgery. Immunomodulating effects of different anaesthetic agents and methods vary and may affect various elements of the immune system. With regard to tumour spread, the most important factor is the function of NK cells. This therefore necessitates a thorough look at the impact of different anaesthetic agents on the count of these cells and their activity.

Volatile anaesthetic agents, including halothane, suppress interferon-induced cytotoxicity of spleen NK cells from mice both *in vitro* and *in vivo* (Marcovic et al., 1993). This effect is mediated by stimulating CD8+ cells activity that results in suppression of the killing capacity of the cells. Volatile anaesthetics exert their influence by the ability to interfere with gene expression and signal transmission. For example, by their ability to decrease intracellular release of calcium they suppress expression of NO-synthetase and thus suppress production of NO in clinically relevant doses (Tschaikowski et al., 2001).

Sodium thiopental, a widely used barbiturate for induction of anaesthesia both in humans and in animals, interrupt the response to antigen stimulation in rats for the period of three weeks (Nishina et al., 1998). Phagocytic capacity of macrophages is also depressed by decreased production of super oxide anions.

Another more modern agent used for induction and maintenance of anaesthesia is propofol. Its immunomodulating effect is due to alterations in the synthesis and secretion of cytokines, reactive oxygen species and other inflammatory mediators. Propofol has such actions because of omega-6-oils, which are constituents of the propofol lipid part (Herr et al., 2000). These oils also bind to zinc and iron ions irreversibly and thus frustrate oxy-reduction reactions and free radicals generations.

Prolonged use of opioids results in suppressed proliferation and differentiation of T-Lymphocytes and enhanced apoptosis of thymus cells in mice (Fuchs & Pruett, 1993). Immunosuppressive effects of the different opioid agents are varied. Morphine suppresses NK cells activity, the response of T-Ly to mitogens, antibody generation by B-Lymphocytes, phagocytic ability of macrophages and polymorphonuclear neutrophils. In spite of this complex immunosuppressive influence, opioids reduce the effect of surgery that increases tumour spread (Page et al., 2001). The impact of meperidine and fentanyl is associated with a decreased production of IL-4 by T-Ly and generation of other cytokines by macrophages. Furthermore, these effects are not mediated by opioid receptors, unlike the actions of morphine. High doses of fentanyl and sufentanyl suppress NK cells activity but the exact role of this mechanism of tumour spread is not fully understood. In some recent studies it was shown that there are opioid-specific binding sites on the lymphocytes, astrocytes, microglia, lung endothelial cells (both normal and tumour) and peripheral nerves. These sites are named  $\mu_3$  receptors. Morphine and methadone interact with  $\mu_3$  receptors but fentanyl does not. Stimulation of these receptors provokes a release of NO by endothelial cells and decreases adherence of granulocytes. Thus morphine and methadone may down-regulate the inflammatory response to surgery but fentanyl does not display such effect. Methadone and morphine are potent inducers of apoptosis in several types of human cancer cells thus resulting in suppressed tumour growth (Maneckjee, 1999). Their apoptotic effect is mediated partially by protein kinase C that down-regulates apoptosis and by the anti-somatostatin action of morphine. On the other hand, acute pain suppresses cytotoxicity of natural killers and thus facilitates tumour spread in animals (Page et al., 2001). However, this result contradicts an earlier study by Greisen

(Greisen et al. 1999). In some other studies was shown that the release of beta-endorphin resulted in enhanced mitogen-induced proliferation of human lymphocytes that is associated with the C-terminal of its molecule (Morley, 1999). Increased activity of NK cells is found during unaided epidural anaesthesia (Bar Yosef et al., 2001; Procopio et al., 2001).

The pain on its own can induce a stress response and thus can modulate the immune function. However, stressors could be surgery and so is anaesthesia. So it is difficult to determine the final effect of the combined influences of surgery, anaesthesia, and pain. Definitely, the metastatic process can be regulated by the stress. The presence of activated oncogenes and/or inactivated tumour-suppressing genes may result in activation of multiple transcription factors. Abnormal activation and interaction between these factors result in unrepresentative expression of metastasis-related proteins. Stress contributes to expression of such proteins and through that participates actively in the biology of tumour metastases (Xie et al., 2003). There are 17-20 hormones and neurotransmitters, which have an immunomodulating ability (Khansari et al., 1990) but the stress can modify immune function in two main ways – the hypothalamus-pituitary-adrenal axis and the direct impact of the sympathetic nervous system on the immune function. Evidence to sustain this assertion is provided by the data presented by Felten & Felten (1991) about the abundant number of sympathetic nerve fibres innervating the lymphoid organs and the presence of beta-adrenergic receptors on the immune cells. Therefore, the immune response could be modified by modulation of beta-adrenergic activity. Resistance of NK cells to the stimulation of beta-adrenergic receptors in the prepubescent rats is the cause for the markedly lower immune response to stress and corresponding lower spread of tumour cells (Page et al., 2000). Reproducing stress in mice using a smell of other stressed mice (a nature and ethology-related model of stress), Moynihan (1994) found that cell-mediated immunity was suppressed in these mice that have been shown with suppressed T-Ly and NK cells activity, decreased production of IL-2 and with enhanced humoral immunity presented with increased antibody response – IgM and IgG. This difference could be due to different activation of Th subtypes. The principal role in this process is played by glucocorticoids, which activate Th1 cells and stimulate them to produce cytokines.

Predominance of Th1 over Th2 cells is associated with lack of protection from cell-mediated immunity and increased spread of tumour cells.

Both hypothermia and hypotension accompanying each anaesthesia scheme disturb immune function either indirectly, by decreasing tissue oxygen concentration and oxygen-dependent killing potential of neutrophils, or directly by decreasing the number and activity of the immune cells including NK cells (Taylor, 1998; Ben Eliyahu et al., 1999). Comparing the effects of different anaesthetic agents on NK cytotoxicity and spontaneous metastases Melamed et al. (2003) found that hypothermia did not exert a significant effect on these parameters. All of the investigated agents in this study, such as ketamine, thiopental, halothane, with the exception of propofol, suppressed significantly NK activity and resulted in enhancement of lung metastases. The differences between effects of these agents have been rather big, as ketamine was shown to have the most noxious impact. These investigators claim also that immunosuppressive and metastasis-enhancing effects of the agents mentioned above can be decreased by prior application of peripheral beta-adrenergic blockade and the use of immunostimulators; that explains the mechanism of their effects.

Another study showed an increased growth of liver metastases under the influence of severe surgical stress in rats, an effect that correlated with the plasma levels of products of lipid peroxidation (Hirai et al., 1997). Application of antioxidants abolished the metastatic effect of this surgical stress. One of the most reactive free radical – the hydroxyl radical ( $\bullet\text{OH}$ ) – can catch electrons from thiols and thus may interact with nitrogen bases of the nucleotide acids. Therefore,  $\bullet\text{OH}$  has the ability to change the genetic information of the cell, since it possesses a potential to generate tumours (Castillo et al., 2001).

Genes controlling the programmed cell death (apoptosis) could play an important role in the development of tumours. For example, some lymphoid tumours are characterised by high gene expression of bcl-2 gene (Kroemer, 1997). This gene codes for an oncoprotein that blocks apoptosis. The ability of such oncoproteins to suppress programmed cell death provides cells with abnormal genes the means to escape from such mechanism that would destroy them. Overexpression of bcl-2 has been shown in neoplastic lymphocytes

(Thompson, 1995). Furthermore, these cells multiply and are in high risk of more gene damage that would inevitably lead to increased malignancy. Antineoplastic properties of the chemotherapeutic agents are due to their ability to induce apoptosis of the tumour cells, either by amplification of the expression of death receptors on the tumour cell surface or by release of mitochondrial cytochrome C (Kaufmann & Earnshaw, 2000). With regard to anaesthetic agents, some studies have considered their effects on apoptosis. These effects would undoubtedly affect along the line tumour metastases positively or negatively. Some anaesthetic agents have been known to exert a prometastatic effect by delaying apoptosis (Wise-Faberowski, 2001; De Klaver, 2002) of the tumour cells or by increasing the apoptosis of cytotoxic immune cells, mostly NK cells (Yamada, 2002; Delogu, 2003) while others have had an opposite antimetastatic effect. Stress, including anaesthesiological stress, increases cell apoptosis (Fumarola & Guidotti, 2004).

Like other studies of this kind this review has shown the various views and counterviews based on experimental studies. However, it is worthy of note to state that the mechanisms of tumour metastasis involve neuroimmunomodulatory network (Horning-Rohan, 1995; Ben-Eliyahu et al., 2000) that is related chiefly to stress-related modulation of the cell-mediated immunity.

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