THE USE OF CISPLATIN IN THE MANAGEMENT OF APPENDICULAR OSTEOSARCOMA IN THE DOG

by

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DEDICATION

To my parents-who have always supported me in my endeavours-my love and thanks.

The great pleasure of a dog is that you may a make a fool of yourself with him and not only will he not scold you, he will make a fool of himself too.

Samuel Butler, (1835-1902)

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The management of patients with osteosarcoma is not undertaken by an individual. Not only is it very time consuming but more importantly it requires input from colleagues who have expertise in the various facets that make up the programme. Consequently I acknowledge the contributions of the following individuals without whose involvement management of these cases would have been an impossibility[1]:

The limb salvage surgery was carried out under the direction of Mr Andrew Miller, Lecturer in Surgery, (114993) and Mr Stuart Carmichael, former Lecturer in Surgery, (110232, 110809, 112509 and 114324). The amputations for case numbers 116799 and 115930 were performed by myself with the assistance of other members of staff and students. Anaesthesia was provided by the House Surgeons under the direction of Dr J. Reid, Lecturer in Surgery. Post operative care was provided by the House Surgeons and the nursing staff. The majority of the routine radiographs were taken by Miss J. Lloyd. The practical organisation of the chemotherapy was by Mrs A. Parker, the Head Surgery nurse, and her staff. The administration of the cisplatin and blood sampling was performed by myself.

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Finally my thanks to Professor N.T. Gorman, Professor of Veterinary Surgery, for advising on the chemotherapy for these cases and supporting this project.

[1]All references to a position as a lecturer and university departments refer to the Glasgow University Veterinary School unless otherwise stated

DECLARATION

I, Thomas James Anderson, do hereby declare that the work in this dissertation is original, was carried out by myself or with due acknowledgement and has not been presented for the award of a degree at any other university.

date: 20TH MAY 1992

SUMMARY

This dissertation presents analysis of various features of cases of canine osteosarcoma (including one case of chondrosarcoma) managed in the Department of Veterinary Surgery, Glasgow University Veterinary School.

These features include:

- the clinical outcome in cases managed with surgery and cisplatin chemotherapy
- experiences with the use of bone allografts for limb reconstruction following en bloc resection
- analysis of certain aspects of the pharmacokinetics of the drug administered to selected patients

These findings are presented in the light of published work on the treatment of osteosarcoma and the use of cisplatin in the dog and man.

The major conclusions are:

- subsets of the tumour may exist, with the potential for varying biological behaviour
- the management of osteosarcoma with cisplatin chemotherapy has a positive benefit on survival, in selected patients, but death due to metastatic disease is almost inevitable
- the design of chemotherapeutic protocols for canine osteosarcoma is not finalised
- though the area under the platinum in plasma ultrafiltrate concentration-time curve gave a consistent value in the clinic at Glasgow University Veterinary School it is not suitable, in isolation, for comparing administration protocols
- the use of massive allografts in conjunction with cisplatin chemotherapy requires further evaluation
- multi-centre prospective trials would be the way to pursue these goals

INTRODUCTION

Osteosarcoma is the commonest primary bone tumour in the dog. It represents approximately 80% of all primary bone tumours with the remaining cases divided principally between chondrosarcoma (10%) and haemangiosarcoma and fibrosarcoma (7% combined) (Goldschmidt and Thrall 1985a). It accounts for between 2-7% of all canine tumours, affecting around 8-10,000 dogs per year, in the USA (LaRue and Withrow 1989). This is high compared to the incidence in the human population (approximately 1000 cases per year in the USA). As a consequence of this high incidence in the dog compared to man, and the similar biological behaviour, it has been identified as an animal model of the human disease (Brodey and Riser 1969, Misdorp 1980, Pierrepoint 1985, Straw and others 1990, Withrow and others 1991).

Osteosarcoma is a devastating condition in both the canine and human patient. It is characterised by the destruction of the local bony architecture with associated dysfunction and early metastasis resulting in a high mortality rate.

Treatment of the condition presents two major challenges for the clinician:

- Control of local disease and maintenance of function
- Control of disseminated disease

It is generally believed that in both species amputation or tumour resection alone is palliative, having no effect on the development of metastatic disease. Few dogs apparently cured by amputation have been reported (Brodey and Riser 1969, Spodnick and others 1991).

Though amputation is acceptable in smaller dogs with a good functional result it is not ideal in the large and giant breeds. In man a number of surgical approaches are advocated. These include amputation or resection and reconstruction with an allograft or endoprostheses.

The use of chemotherapeutic agents has shown beneficial effects in delaying of development of metastatic disease in man have been shown (Yasko and Lane 1991). These techniques have not proved as useful in the dog (Madewell and others 1978, Jeglum 1985). However, some beneficial effects have been demonstrated. Currently the drug receiving most attention for the treatment of osteosarcoma in the dog is diamminedichloroplatinum (II) (cisplatin).

CHAPTER 1.

TUMOURS OF THE SKELETAL SYSTEM OF THE DOG: REVIEW OF CLASSIFICATION AND BEHAVIOUR WITH AN OVERVIEW OF MANAGEMENT IN MAN AND THE DOG

PRIMARY SARCOMAS OF BONE

A sarcoma is defined as a malignant neoplasm derived from mesenchymal tissues (Dubielzig 1989). These tissues are derived from the mesoderm, which is the third germ layer of the embryo. Mesoderm gives rise to the blood and lymphoid tissues and also to the connective tissues including the general supportive tissues (fibrous and adipose) and the more specialised tissues (bone and cartilage) (Leeson and Leeson 1979). Bone is an organ of connective tissues. These cell types range through the general supportive connective tissues (fibrous and adipose), haematological tissues (endothelial and and cell precursors) to the more specialised connective tissues of bone and cartilage (Pool 1990).

Nine basic types of primary bone sarcoma are described. These are summarised in Table 2 (page 3). Osteosarcoma is heterogeneous in nature. A tumour containing neoplastic osteoid, whether mineralised or not, is by definition an osteosarcoma. However, there may be significant proportions of other matrix products such as neoplastic cartilage within the tumour mass. Oteosarcoma is classified depending on the proportion of cell types and matrix it contains (Pool 1990) (Table 1, page 2). The distribution of osteosarcomas within the canine skeleton is summarised in Figure 1, (page 4). Certain regions are over represented.

Osteosarcoma type	Characteristics of histopathology
osteoblastic	predominantly neoplastic bone
chondroblastic	predominantly neoplastic cartilage
telangiectatic	cells produce osteoid but there are attempts at the development of vascular channels (resembling haemangiosarcoma)
fibroblastic	neoplastic fibrous stroma with little osteoid or neoplastic bone
combined type	mixed matrix but no dominant pattern
poorly differentiated	cell morphology difficult to classify but tumour osteoid and bone present

Table 1. Subdivision of osteosarcomas by nature of matrix.

Tumour classification	Distinguishing features
osteosarcoma	cells produce osteoid, not necessarily mineralized
chondrosarcoma	characterised by the production of neoplastic cartilage, there may be areas of calcification these are not areas of osteoid bone also may develop in chondrosarcomas by endochondral ossification (Pool 1978)
fibrosarcoma	cells produce varying amounts of collagenous matrix
haemangiosarcoma	areas of undiffereniated sarcoma with areas of primitive vascular channels
liposarcoma	fat may be demonstrated within the tumour cells
undifferentiated sarcoma	cell types very primitive with insufficient organization to produce recognisable matrix
giant cell tumour or osteoclastoma	large numbers of giant cells care must be taken as giant cells are a common feature of the histopathology of many bony lesions including osteosarcoma and also of some benign lesions (Pool 1978)
myeloma	population of neoplastic plasma cells
lymphoma (previously referred to in the literature as reticulum cell sarcoma, Jeglum 1985)	population of neoplastic lymphocytes

Table 2. Classification of primary bone sarcomas in the dog.

The type of sarcoma found varies with the region of the skeleton. Chondrosarcoma is far commoner in the axial skeleton than the appendicular and for osteosarcoma the converse is true. It is more difficult to generalise about the other tumours because of their infrequent occurrence. Sarcomas may arise in any part of the bony structure. However, in the canine long bone they almost exclusively originate in the medullary cavity (Pool 1990).

Rare periosteal or juxtacortical (arising from the periosteal region) osteosarcomas and chondrosarcomas are described (LaRue and Withrow 1989).

Sarcomas arising from bones of the skull have posed a problem for pathologists in classification. Some benign conditions can be confused with neoplasia (e.g., craniomandibular osteopathy, Riser and Newton 1985) by inexperienced veterinary or uninitiated non-veterinary pathologists.

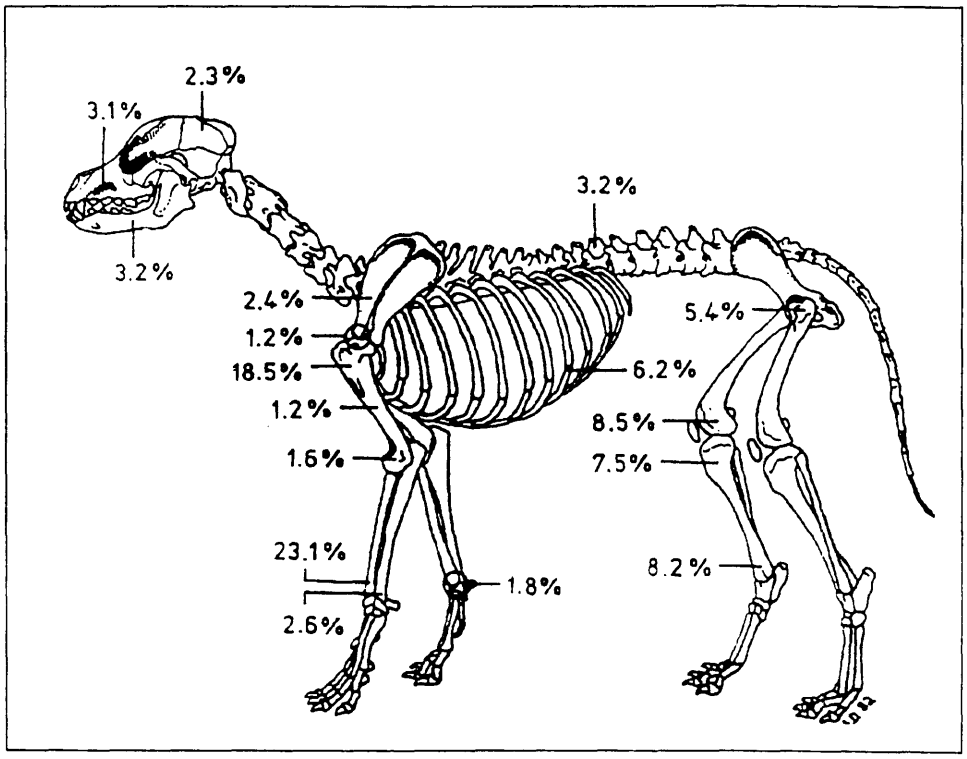


Figure 1. Distribution of osteosarcoma in the canine skeleton from the records of 1215 cases (Goldschmidt and Thrall 1985a).[1]

True osteosarcomas of the skull do occur albeit infrequently (Figure 1, page 4). A morphologically distinct tumour occurs in the skull region alone that has a distinctive multilobulated pattern. This has been referred to by many names in the past (e.g., canine aponeurotic fibroma, chondroma rodens, multilobular

[1]Illustration reproduced with permission of the publishers. In: Textbook of Small Animal Orthopaedics (1985) (Eds. Newton and Nunamaker), Philadeplphia, J.B. Lippincott and Company, page 888, Figure 74-1.

osteoma and chondroma Straw and others 1989). Currently the tumour is referred to as multilobular osteochondrosarcoma by Straw and others (1989) but Pool (1990) suggests the term multilobular tumour of bone. Though it has similarities with appendicular osteo- and chondrosarcoma there are differences in cell morphology and behaviour (Pool 1990). Though usually described as benign, a recent review has suggested metastasis in 58% of cases and it is perhaps better thought of as a malignant tumour (Straw and others 1989).

FUNCTIONAL CLASSIFICATION

The classic histological descriptions of osteosarcoma give a description of the cellular components of the tumour. However, the behaviour is not well correlated with the basic histological appearance (Rosen 1987). Histological diagnosis alone is limited in that it does not take account of the extent of the disease in the patient.

Several methods have been developed to describe and stage neoplasms in an effort to improve management decision making and refine the prognosis. These methods include other features of the tumour and the patient as a measure of progression. The World Health Organisation has developed the TNM system. This was originally developed for the classification of human tumours to allow meaningful comparison between the work of different clinical centres. It is known as TNM system because it is based on recording features pertinent to the tumour, lymph nodes and assessment of the extent of metastasis.

T	stage of primary tumour
N	stage of local lymph nodes
M	the absence or presence of distant metastasis

The important features of this system in relation to bone tumours in dogs are shown in Table 3 (below).

	Grade	Description
Tumour	T0	no evidence of tumour
	T1	tumour confined within the medulla and cortex
	T3	tumour extends beyond periosteum
Metastasis	M0	no evidence of distant metastasis
	M1	distance metastasis detected

Table 3. World Health Organisation staging criteria for skeletal sarcomas[2]

[2]Owen 1980, quoted by Withrow and McEwan 1989.

The local lymph node status is not described for this tumour because of the low rate of metastasis to regional lymph nodes (<5%, Pool 1990). Lymph nodes, however, may be increased in size in response to the inflammatory reaction associated with the tumour (Straw and others 1991). Invasion of the lymphoid system occurs late in the disease and is thought to be of little clinical importance (Pool 1990). In man, this is thought to be a result of the lack of lymphoid drainage of the intramedullary bone (Robertson and others 1984).

A staging system more specific to musculoskeletal tumours was described by Enneking for use in humans (Table 5, page 8). Evaluation of this system in humans has demonstrated reasonable correlation with long term prognosis (Lane and Glasser 1987). This system has been used in the veterinary literature for the description of patients but has not yet been related to outcome. Some authors have the subjective opinion that there may be differences between the behaviour of the morphologically classified canine osteosarcomas (Misdorp 1980, Gleiser and others 1981) in the way there appears to be in humans. However, the consensus is that a relationship has yet to be demonstrated in the dog (Straw and others 1990). In man classification of bone sarcomas on histological grounds has been further advanced by dividing them into three broad categories (Table 4) (Rosen 1987).

Cell type	Histological appearance
small cell	small, round, undifferentiated
spindle cell	more differentiated with spindle appearance
pleomorphic	elements of the other two categories

Table 4. Categories of cell type used to classify sarcomas in man (Rosen 1987)

This is irrespective of the conventional histological subgroups. Use of this system assists with therapeutic decisions and correlates with the variation in behaviour that is observed.

These tumour cell types broadly exhibit common behaviour and response to therapy. This is summarised in Table 6 (page 9). The range of bone pathology in the dog is limited compared to man, with small cell carcinomas of bone in particular being very rare (Brodey and Riser 1969).

Examples of small cell sarcomas in man are Ewing's sarcoma (not recorded in the dog), some forms of osteosarcoma (rare), chondrosarcoma, liposarcoma and neuroblastoma. That these unusual osteosarcomas are not aberrant forms of Ewing's sarcoma was confirmed by Fellinger and others (1991) using immunohistochemical techniques.

Canine tumours are not routinely classified according to this system at GUVS or apparently in the general veterinary oncological literature. However, it is felt that this system could be adapted to the classification of sarcomas in domestic animals though it is not certain that it would prove to be as useful (McCandlish[3], personal communication).

Osteosarcomas are often described in some of the veterinary literature as being of high or low grade. This distinction between high and low grade is a subjective assessment based on cell morphology, frequency of mitotic cells and the level of cell differentiation. The more pleomorphic, the greater the numbers of mitotic cells and the less well differentiated the cells are the more aggressive or high gade the the tumour is assessed to be. Most canine tumours are described as moderately or severely anaplastic (Withrow and others 1991).

Flow cytometry, a technique that quantifies the amount of DNA in cells, can be used to subdivide tumour types. Using this technique, subsets of human osteosarcoma have been shown to have different metastatic potential. The more deranged the DNA content the less likely was metastasis (Mankin 1987a). On analysis, human and canine osteosarcomas show similar characteristics (Withrow and others 1991). Analysis of canine osteosarcoma and its metastatic deposits have shown that metastatic clones may be derived from more than one cell (Fox and others 1990).

[3]Dr. I.A.P. McCandlish, Department of Veterinary Pathology, Glasgow University Veterinary School

Histological grade	Compartments involved	Metastasis	Grade
G1	T1	M0	IA
	T2	M0	IB
G2	T1	M0	IIA
	T2	M0	IIB
G2	T1	M1	IIIA
	T2	M1	IIIB

Table 5. Enneking system for the staging of malignant musculoskeletal sarcomas.

Histological grade	G1-low grade; G2-high grade
Number of compartments involved	T1-confined to one compartment; T2-extendended to other compartments
Metastasis	M0-no metastasis; M1-metastasis to distant site
Grade	related to combination of these factors

Benign tumours are graded: 1 latent; 2 active; 3 aggressive

Cell type	Biological behaviour	Response to therapy
spindle cell	behaviour further differentiated into low grade and high grade	usually radio resistant more resistant to the common chemotherapeutic protocols
	high grade: malignant, likely to metastasise by haematogenous route	
	low grade: low malignancy, metastasis uncommon	
small cell	highly malignant and likely to metastasise usually by haematogenous route	radiosensitive sensitive to common chemotherapeutics
pleomorphic	highly malignant and likely to metastasise by the haematogenous route	may be resistant to radiotherapy may be insensitive to common chemotherapy protocols

Table 6. Behaviour and response to therapy of human bone sarcomas based on cell morphology

Newer techniques involving the use of monoclonal antibodies are becoming available for use in the classification of sarcomas[4]. These should help to define the origin of these tumours by enabling one to work back from the tumour to the original cell type. They also may help in the classification of sarcomas into groups that have similar responses to therapy and prognosis by identifying related types.

BEHAVIOUR OF OSTEOSARCOMA

Local

Initially the clinical picture is of local inflammation with heat, swelling and pain over the affected region. This often presents as an acute exacerbation of a chronic, progressive, low grade lameness. Initially swelling may be minimal and with the more proximal tumours may be very difficult to appreciate.

Local effects are at first destruction of medullary and cortical bone. As the tumour extends through the cortex the periosteum is stripped from the bone surface, contributing to the pain experienced (LaRue and Withrow 1989).

Surrounding the neoplastic area there is an area of intense inflammation, which presumably also contributes to the discomfort. Extension into the joint is rare but will develop occasionally in long standing tumours.

Pathological fracture, as the result of the weakening of the bony architecture, may occasionally occur. Where there has been acute exacerbation of a relatively low grade lameness this may be related to local micro-fractures. The term "pathological fracture" is usually retained for full cortical disruption. Fracture in cases of tumours of the axial skeleton may result in the sudden onset of neurological signs following collapse of the vertebrae and spinal cord compression.

Animals with long standing metastatic pulmonary disease may develop hypertrophic pulmonary osteopathy - a proliferative disorder of the periosteum. Though the aetiology is uncertain this is thought to be mediated by a neurovascular mechanism as it can be treated successfully by vagotomy (Ogilvie 1989).

Distant

Osteosarcomas are recognised to be metastatic. The primary route of metastasis is haematogenous, probably via the loose endothelial junctions in the bone marrow sinusoids (Pool 1990). Metastasis in the first instance is usually to the lungs. Metastasis to regional lymph nodes occurs rarely. The incidence of pulmonary metastasis is 10% based on radiographic evaluation and 45-65% at post mortem examination (Pool 1990). Lui and others (1977) described the incidence of pulmonary metastasis at post mortem as 9/73 cases (12%) (the timing of these

[4]Professor N.T. Gorman, Department of Veterinary Surgery, Glasgow University Veterinary School

examinations ranged from the time of diagnosis to 25 months post surgery). Interestingly about half of the metastatic deposits mimic the physical appearance of the primary (Pool 1990).

The incidence of metastasis to other organs in untreated animals is low (Pool 1990). Metastasis to the other parenchymatous organs (liver, kidney, spleen) is occasionally observed. Metastasis to other areas is rare. There is one case report of metastasis to the eye (Render and others 1982).

Metastasis to bone is unusual in untreated cases (Pool 1990). Lui and others (1977) observed this in only 3/73 of their cases. Assessment with other techniques has revealed an instance of 3-19% (scintigraphy, Lamb 1991) and 6.4% (radiographic skeletal survey, Straw and others 1990).

This distribution may be a function of time. Presumably as the tumour cells are released into the venous circulation the majority will become lodged in the pulmonary tissue (either physically or in relation to the mononuclear phagocyte system).

Animals that undergo management by amputation alone demonstrate a higher rate of metastasis to other organs (Brodey 1965, Brodey and Riser 1969, Brodey and Abt 1976, Lui and others 1977). The reason for this is uncertain. The primary is usually controlled adequately by removal of the affected quarter. The development of these aberrant deposits, after the removal of the primary, suggests that they were present before amputation or that they represent spread from pulmonary metastasis.

The pattern of metastasis may vary with the region of the skeleton. Osteosarcomas of the skull are described as having low rates of metastasis (Pool 1990). Parosteal sarcomas, though rare are thought to metastasise late (LaRue and Withrow 1989). Misdorp (1980) feels those of the hind limb are relatively more aggressive.

The other primary sarcomas of bone are also locally aggressive and metastatic but the patterns vary. Their relative rarity makes establishing their behaviour difficult. They are summarised in Table 7 (page 12).

Myasthenia gravis has recently been reported in association with osteosarcoma (Moore and others 1990) in three cases. Myasthenia gravis as a para-neoplastic syndrome in cases of thymoma is well-documented in the dog and man (Crow 1989). One of the reported cases associated with osteosarcoma had received an intensive course of cisplatin chemotherapy and another developed myasthenia 5 years before the tumour. It is difficult to attribute either of these cases directly to the tumour. If myasthenia is truly a problem, it may become more prevalent as the management of cases changes.

Tumour	Behaviour
chondrosarcoma	clinical course tends to be longer with late and lower rate of metastasis (Lui and others 1977)
haemangiosarcoma	very destructive before clinical signs develop often with metastasis at the time of diagnosis
fibrosarcoma	relatively rare as a primary bone tumour but there is an impression that it metastasises late in common with other fibrosarcomas

Table 7. Behaviour of the central non-osteogenic primary bone sarcomas compared to osteosarcoma.

SIGNALMENT OF DOGS DEVELOPING OSTEOSARCOMA

The signalment of the reported cases of osteosarcoma are summarised in Table 9 (pages 16-17). The majority of animals affected by osteosarcoma are the medium, large and giant breeds of dog. The largest survey is of 1215 cases (collected by Brodey, analysed by Kisler and quoted by Goldschmidt and Thrall 1985a). In this survey 95% of affected individuals were 14kg or greater in weight. This weight distribution has been observed by other authors. There was a slight preponderance for males though this may be breed related (in Saint Bernards this appears to be reversed).

Certain breeds appear to be over represented including the Irish setter, Saint Bernard, Great Dane, rottweiler, and German Shepherd dog. Some of this may be related to body size, however, a familial tendency has been demonstrated in some breeds (see Table 11, page 19) and these breeds have also been over represented in some other surveys. The distribution of tumours also appears to be related to body size with tumours of the axial skeleton being a feature of small breeds (Table 9, page 18).

RISK FACTORS AND AETIOLOGY

The aetiology of primary bone tumours is unknown (LaRue and Withrow 1989). However, examination of the circumstances in which primary bone sarcomas are found has identified some common features (Table 11, page 19).

Primary bone tumours can be induced by irradiation either experimentally or as a consequence of radiotherapy for another neoplasm (Thrall and others 1983). Experimental exposure to radioactive compounds may also induce

osteosarcomas (LaRue and Withrow 1989). This is of particular interest as strontium, one of the implicated elements, is a feature of radioactive fall out and is concentrated in the growing bones of young animals and children. There is no evidence that radiation is a factor in spontaneous disease (Owen 1986).

A number of family lines of rottweilers and Saint Bernards have been identified with a higher than expected incidence of osteosarcoma development. It could be speculated that there was vertical transmission of an infectious factor. Alternatively, a heritable inability to suppress osteosarcoma development is possible, though excessive tumour development in general was not a reported feature of the affected animals. Another possibility is a heritable abnormality of the affected cell line(s) resulting in more frequent malignant transformation.

Osteosarcoma has been transmitted experimentally by injection of puppies with tumour cells (LaRue and Withrow 1989). This led to speculation of a viral aetiology though this has not been demonstrated. Type C virus particles have been demonstrated in naturally occurring giant cell tumours in cats (McGlennon 1991). Their significance is unknown and these tumours are exceedingly rare.

Most spontaneous osteosarcomas, and sarcomas of the medullary canal, occur in the region of the metaphyseal scar. Though this was previously an area of intense bony activity, in the typical canine osteosarcoma patient it is quiescent. In the typical human patient it is still an active area. In an examination of normal growth of the distal radius and ulna in Great Danes, isolated islands of growth plate cartilage were found embedded in the growing diaphyseal bone (Riser and Shirer 1965). Their fate is unknown but these "unfulfilled" cells are suggested as a potential source of neoplasia (Owen 1986). It is feasible that cartilage cells that developed during the reparative phase of fracture healing also become isolated within bone may undergo malignant transformation (Bennett and others 1979).

Chronic trauma is another possible explanation for the metaphyseal distribution of osteosarcoma (Pool 1990). Evidence for this appears to be:- the prevalence in large and giant breeds; the ratio of weight carried between the fore and hindlegs is similar to the ratio of tumour development; the low incidence in the axial skeleton. This seems to be circumstantial evidence with no objective evidence to support it.

The sporadic occurrence of osteosarcoma after fracture is interesting. The prevalence following fracture is not known and would be difficult to quantify accurately. However, it happens frequently enough to be of concern. Affected bones had not necessarily been treated surgically. There are a number of features of fracture healing and management that appear to be related to osteosarcoma development (Table 8, page 15).

Tumours occurring at fracture sites are unlikely to be sporadic naturally occurring tumours as their distribution is different to that of the spontaneous

disease (they are diaphyseal rather than metaphyseal). The lag to clinical disease (approximately 5 years) is such that the majority could not have been present at the time of fracture (Stevenson 1991).

It is possible that fracture associated sarcomas are related to more than one of the potential factors. Obviously release of metal ions cannot be related to all instances as sarcomas can develop without internal fixation. Many cell types of varying potentials are attracted to fracture sites and conceivably these may become trapped within the healing bone. There is often a disturbed pattern of healing with instability sometimes associated with infection (Stevenson 1991). Chronic inflammation has been implicated in oncogenesis of other tumours -e.g., squamous cell carcinoma (McEwan 1989a). The other feature of these areas of bone will presumably be an increased population of pleuripotential cells and the various substances that stimulate their differentiation.

It is likely that there is more than one pathway to primary bone sarcoma development and that the cells that undergo transformation are of similar type and degree of differentiation.

BENIGN TUMOURS OF BONE

Benign tumours of the skeleton are rare in dogs (Pool 1990) but a number are recognised and these are summarised in Table 12 (page 20). They are of little clinical significance, unless they impinge on surrounding tissues, but may be of importance in differential diagnosis.

TUMOURS INVADING BONE

Bone may be directly invaded by tumours developing in surrounding tissues. These may alter the bony structure and cause bony pathology. A number of such tumours are recorded and these are summarised in Table 13 (pages 21-23). These tumours may be metastatic as well as being locally aggressive.

Bone may also be the site of metastasis from distant tumours, though this is unusual and has a lower incidence in the dog compared to man (Pool 1990). However, this may reflect the fact they are often difficult to find and are generally not aggressively searched for. Metastases from most types of tumour have been recorded with the commonest being carcinomas of the mammary gland, liver, lung and prostate (LaRue and Withrow 1989). The bones most affected are the humerus, femur and vertebral column It is suggested that the areas that are most likely to be affected have high vascularity (Pool 1990).

Factor	Possible significance
chronic presence of metal in the body	1. accumulation of metals in remote tissues has been identified 2. senitisation of immune system 3. many of the metals used in implants have been demonstrated to be oncogenic in experimental animals including chromium, cobalt and nickel 4. long term implants (hip prostheses) possibly linked to increased rate of lymphoreticular disease in man
implant corrosion	1. an implant associated with known corrosion problem (Jonas pin) was associated with a very high incidence of sarcoma formation and osteomyelitis (Sinbaldi and others 1982) 2. there are sporadic case reports of sarcomas involving bone in dogs (Rosin and Rowland 1981) and soft tissues in humans related to corroding metallic foregn bodies 3. modern implants do not corrode visibly but there is a degree of corrosion in the region of the screw/ plate interface
altered cellular activity	1. fracture repair involves complex and evolving changes in local cellular activity with many fairly undifferentiated cell types and also the differentiation of cell types 2. fracture areas can become areas of chronic inflammation if infected or unstable

Table 8. Factors that have been identified as having a possible role in post fracture sarcoma development (Stevenson 1991).

Author	Date	Number of animals	Sex distribution (ratio male:female)	Age distribution (years)	Weight distribution (kg)	Distribution of lesions	Distribution of breeds
Brodey and Riser	1969	194	1.2: 1.0 (186 dogs)	mean = 7.74 median = 7	96% > 11	appendicular skeleton = 152 (78%): forelimb = 98 hindlimb = 54 axial skeleton = 42 (32%): head = 24 ribs = 8	Five breeds accounted for 123/194 cases: boxer, Great Dane, German shepherd dog, Irish setter, Saint Bernard
Brodey and Abt	1976	65	1.5:1.0	median = 6 (mean not stated)	60/65 (92%) > 16	appendicular skeleton only forelimb = 36/62 hindlimb = 26/62	cross bred = 11 Saint Bernard = 10 boxer = 8 German shepherd dog = 7 great dane = 4 doberman pinscher = 4 Standard poodle = 3 Irish setter = 3 1 representative each of a number of large and giant breed dogs

Table 9. Signalment of reported cases of osteosarcoma in the dog.

Author	Date	Number of animals	Sex distribution (ratio male:female)	Age distribution (years)	Weight distribution	Distribution of lesions	Distribution of breeds
Goldschmidt and Thrall (Brodey analysed by Kisler)	1985	1215	53%: 47% this ratio varies with breed and size	mean=7.5 varies with weight peak for giant breeds 4-7	95% > 14 kg	Appendicular skeleton = 82% Axial skeleton = 18%	relative risk: Saint Bernard 12.77 great dane 7.27 golden retriever 5.27 Irish setter 4.34 doberman pinscher
Smith and Sutton	1988	69	NA	mean=7-8 (10%<=3)	large breeds	Appendicular skeleton=71% forelimb=23/47 hindlimb=24/47	Primarily large breeds: great danes dobermans German shepherd dogs
Spodnick and others	1991*	162	1.1:1.0	mean=7.9 median-8	All >20kg	appendicular skeleton only: forelimb = 105 hindlimb = 57	Not reported

Table 9 (cont). Signalment of reported cases of osteosarcoma in the dog.

*In press

Patient size (kg)	Percentage of 1215 cases	Position of tumour (%)	Sites of involvement (%)
>41	29	appendicular=95 axial=5	distal radius=42 proximal humerus=15
>27 and <41	55	appendicular=79 axial=21	proximal humerus=19 distal radius=14
>14 and <27	11	appendicualr=67 axial=33	proximal humerus=18 distal radius=10 proxiamal tibia=9
<14	5	appendicular=41 axial=59	

Table 10. Distribution of osetosarcoma as a function of size (Goldschmidt and Thrall 1985a).

Factorst	Comment
patient size	The majority (95%) are found in dogs >14kg (Goldshmidt and Thrall 1985a).
sex	slightly commoner in males, possibly related to males being heavier
age	mean age around 7 years with a peak in younger dogs (large/giant breeds which "age early", Brodey 1969, Goldshmidt and Thrall 1985a) youngest recorded patient was a 6 month old bloodhound (Philips and others 1986).
predilection for metaphyseal sites	classically in the large breed dogs these are found at the distal radius, proximal humerus, distal femur and distal tibia; smaller dogs tend to have tumours of the axial skeleton (Goldshmidt and Thrall 1985a).
occurrence after fractures	many reports of tumours following fractures associated with different types of fixation including closed management (Bennett and others 1979, Stevenson and others 1982, Stevenson 1991) including a case following fracture management with an allograft (Vasseur and Stevenson 1987)
occurrence in association with chronic irritation e.g. osteomyelitis and electrolytic reaction	e.g. Jonas pin. This device was developed for the repair of long bone fractures. All cases, in a series of 11, in which it was utilised developed either osteomyelitis or osteosarcoma. Some of these may have osteomyelitis prior to the development of osteosarcoma. A major feature of the device seems to be its propensity to undergo corrosion in a biological situation. A number of cases of instances in humans also seem related to corrosion of implants (Sinbaldi and others 1982). case report of a corroded metallic foreign body with concurrent infection being related to an undifferentiated sarcoma involving the sternum (Rosin and Rowan 1981).
familial and breed incidence	demonstrated in Saint Bernards (Bech-Neilson and others 1978) and rottweilers (Misdorp 1980) some breeds over represented in some series e.g. Irish setters (Lui and others 1977, Maudlin and others 1988)
infarcts of bone	sporadically reported in the literature (Dubielzig and others 1981, Prior and others 1986) and probably of limited importance
radiation	experimental and as complication of radiotherapy for tumours

Table 11. Factors associated with the development of osteosarcoma in the dog.

Tumour	Behaviour
osteoma	slow progressive growth over months to years. no metastasis or malignant transformation reported
endchondroma	benign medullary cartilaginous tumour no metastasis or malignant transformation reported in animals but is recognised in man
multiple cartilaginous exostoses	developmental disease. mono- or polystotic cartilage tipped excrescences protruding from the bone. growth likely to stop with skeletal maturity. malignant transformation likely in animals with multiple lesions. demonstrated to be hereditary in nature (LaRue and Withrow 1989).
bone cyst	rare in dogs . cysts are fluid filled and may be monostotic or polystotic in distribution. aneurysmal bone cysts are very rare in the dog(Goldschmidt and Biery 1985).
fibrous dysplasia	rare in dogs characterized by replacement of cancellous bone by a fibro-oseous matrix (Pool 1990)
ameloblastoma (formally adamantinoma)	rare in the dog derived from the Rests of Malassez (White 1991)

Table 12. Benign tumours and similar lesions of bone in the dog.

TUMOUR TYPE	INCIDENCE	AREA COMMONLY AFFECTED	BEHAVIOUR	MANAGEMENT	PROGNOSIS
fibrosarcoma	rare as a medullary primary sarcoma of bone (Goldschmidt and Thrall 1985). more common as periosteal tumour with local invasion of the bony structure.	mandible, maxilla, digits, axial skelton	generally local infiltration and low rate of metastasis. anaplastic tumours have rapid growth and are likely to metastasise	local wide excision no evidence that chemotherapy worthwhile (LaRue and Withrow 1989)	risk of local recurrence following inadequate excision
synovial sarcoma	rare tumour. mesenchymal in origin and thought to mimic rather than arise from the synovial membrane (LaRue and Withrow 1989)	joints, usually distal, commonly carpus, elbow and stifle	local infiltration and metastasis should be considered as an aggressive tumour (McGlennon and others 1988)	amputation recurrence with local excision is a risk single report of a tumour managed successfully with chemotherapy (Tilmant and others 1986) though one might expect this type of tumour to be relatively resistant to chemotherapy.	amputation may be curative if diagnosis reasonably early but prognosis guarded the use of adjunctive chemotherapy to manage potential metastatic disease has not been assessed in the dog (McGlennon and others 1988)
melanoma	relatively common in oral cavity (Withrow 1989)	mandible, maxilla and digits	local infiltration with significant risk of metastasis (Withrow 1989)	local wide excision	risk of recurrence with local excision. Tumours originating from mucocutaneous junctions and the digits tend to be aggressive

Table 13. Tumors that locally invade bone.

TUMOUR TYPE	INCIDENCE	AREA COMMONLY AFFECTED	BEHAVIOUR	MANAGEMENT	PROGNOSIS
squamous cell carcinoma	common tumour	oral cavity and digits	local infiltration with subungal tumours and those of the digits likely to metastasise	local excision cisplatin may be of some value in the face of disseminated disease (Knapp and others 1988)	guarded prognosis; approximately 30% of cases have local recurrence or metastasis (Susaneck and Withrow 1989)
malignant histiocytotosis	rare has been classified under various names including malignant fibrosarcoma in humans (Rosen 1989). more of a problem with the skeleton in man than in the dog.	two manisfestations malignant and systemic. Identified as an inherited problem in Bernese mountain dogs (Moore and Rosin 1986).	if invades bone causes local destruction	possible response to chemotherapy (too few cases for assessment)	prognosis poor
giant cell or osteoclastoma	very rare	not established	local destruction	in humans responds well to amputation	not established in the dog though long term survival has been reported following amputation (LaRue and Withrow 1989)
rhabdo-myosarcoma	not established because rarely invades bone (Lui and others 1977)	malignant tumours of striated muscle	metastasis likely	likely to recur after excision in the skeletal muscles because of local infiltration	poor

Table 13 (cont). Tumors that locally invade bone.

TUMOUR TYPE	INCIDENCE	AREA COMMONLY AFFECTED	BEHAVIOUR	MANAGEMENT	PROGNOSIS
basal cell carcinoma (acanthomatous epulis)	relatively common	oral cavity basal layer of gingival epithelium	locally invasive metastasis does not occur	will recur if not adequately resected	good with adequate surgical management

Table 13 (cont). Tumors that locally invade bone.

INVESTIGATION OF BONE TUMOURS

Bone lesions require further investigation for definitive diagnosis. The techniques used primarily are radiography and biopsy. Scintigraphy is also potentially of value.

RADIOGRAPHY

Currently radiography is the key technique for investigation of skeletal disease in the domestic species. Though other techniques (e.g., Computer Assisted Tomography-CAT scan, Magnetic Resonance Imaging-MRI) are available, their use is restricted by considerations of economics and access.

Plain radiography will reveal changes in bony architecture and soft tissues that imply the presence of neoplasia Figure 2 (page 26). These are described in Table 14 (page 25). Anatomic position is significant in that classically, though not invariably, tumours develop at certain sites (see Figure 1, page 4).

The radiographic features observed are not exclusive to neoplasia as they are indicative of bone's general response to inflammation and erosion. The most important differentials in the dog are summarised below:

- osteomyelitis
- response to trauma
- fungal infections (in specific geographic regions)

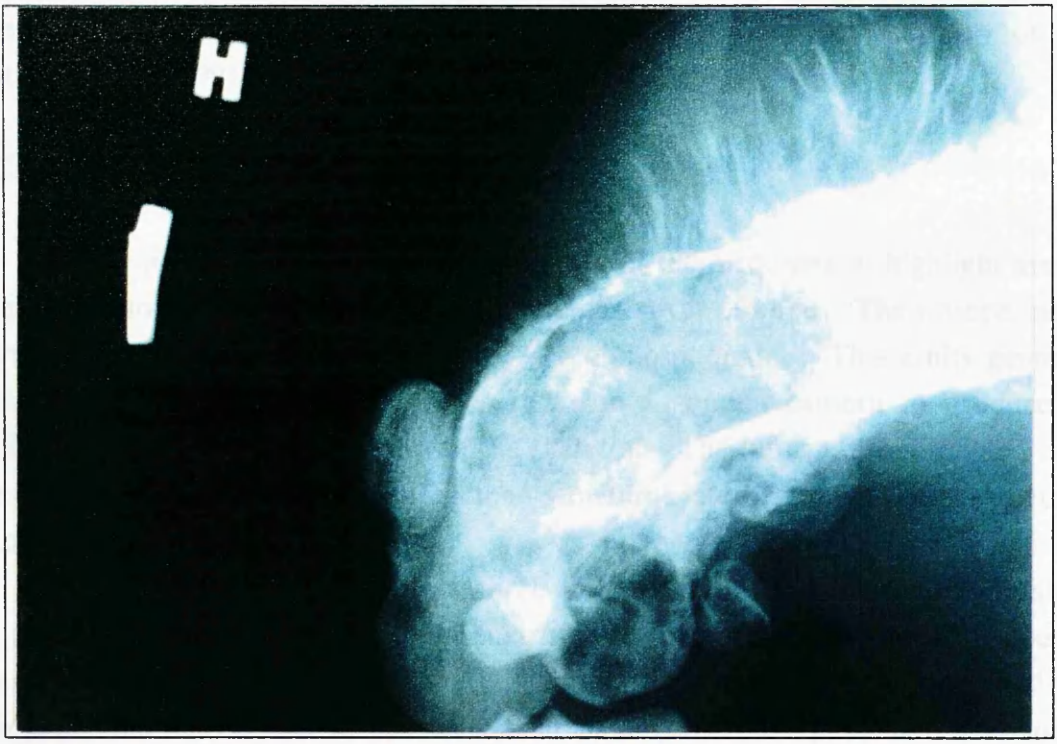
Classic radiographic appearances for the different sarcomas have been described. Current advice is strongly against making a definitive diagnosis based on the radiographic appearance alone because the radiographic findings are not consistent (Houlton 1984, Thrall and Goldschmidt 1985b). For example in a series of 75 appendicular osteosarcomas, reviewed at Bristol Veterinary School, only 61% demonstrated the typical appearance (Gibbs and others 1984). In the UK, however, where radiographic findings and clinical situation are appropriate the index of suspicion is high. Radiography is also used in the assessment of the thorax for pulmonary metastases. Chances of success in recognising early metastatic disease are enhanced by:

- radiographing the chest from both lateral aspects
- inspiratory radiographs

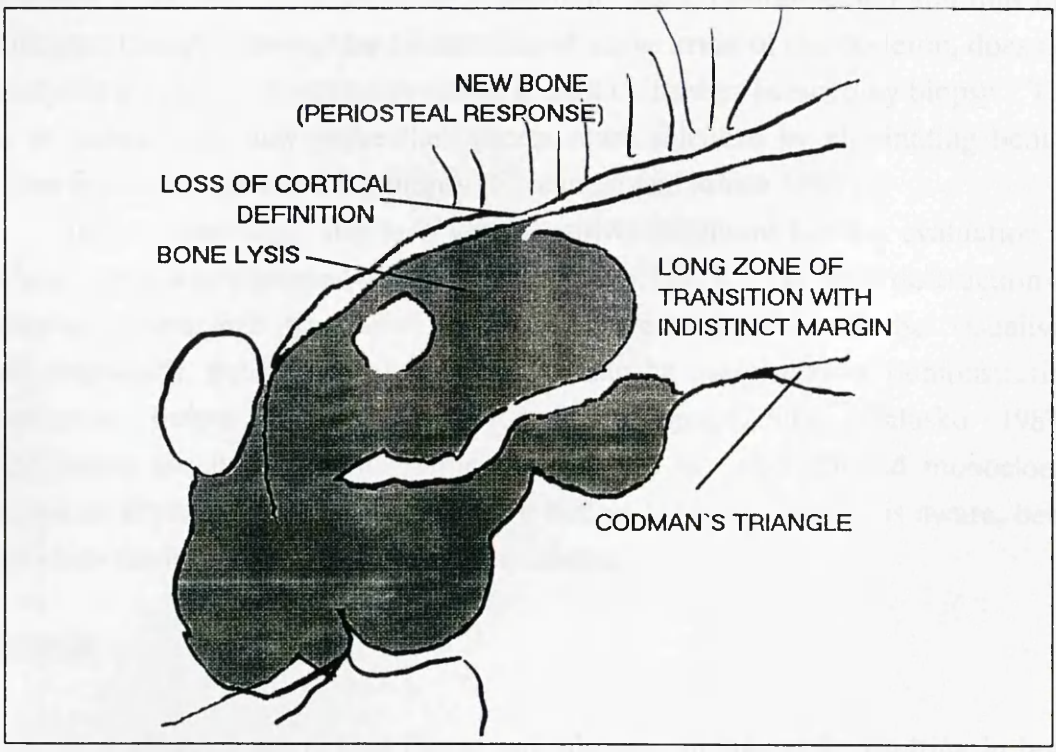
The earliest identifiable lesions are approximately 5mm in diameter (Dennis 1991).

Bony activity	Radiographic change
destructive bony changes	loss of trabecular detail, loss of cortical integrity
productive bony changes	periosteal proliferation, production of new bone, sclerosis
Codman's triangle	the rapidly growing tumour passes through the cortical bone and lifts the periosteum. A feature of the periosteum's response is calcification. This forms a line divergent from the cortex (see Figure 2, page 26) it is not specific to bone tumours (Keally 1987)
soft tissue swelling	increase in density and distortion of local soft tissue structures
poorly defined margins	difficult to define interface between normal and abnormal bone
long zone of transition	along with poorly demarcated margins the amount of bone with an unusual appearance is also greater than with more benign lesions

Table 14. Radiographic changes observed with primary bone tumours



Osteosarcoma of the distal femur



Tracing with description of radiographic changes

Figure 2. Radiographic appearance of osteosarcoma.

Radiographic skeletal survey is advocated by Straw and others (1990) as their technique of choice for the detection of skeletal secondaries though, if available, scintigraphy is advocated as being the technique of choice by other authors (Lamb 1991).

SCINTIGRAPHY

This imaging technique involves the use of radioisotopes to highlight areas of the skeleton with increased blood flow and turn over of bone. The isotope used in the assessment of the skeleton is $^{99\,m}Tc$ diphosphonate. This emits gamma radiation as it decays, which is measured with a gamma camera to produce a representation of the selected part of the skeleton. The information obtained can be considered as a functional rather than structural interpretation of the skeleton (Lamb 1991).

The isotope is distributed in three phases. The first phase is a passive distribution to areas of increased vascularity. In the second phase, the isotope is distributed to the extracellular fluid. In the third phase, the label is attached to the available bone crystal surface. Thus areas of active resorption and new bone production will be highlighted. However, many benign lesions also result in increased uptake (for example spondylosis deformans, osteomyelitis) and thus this technique, though showing the distribution of active areas of the skeleton, does not specify their nature. Suspicious regions should be further pursued by biopsy. The use of radiography may make the process more selective by eliminating benign lesions from those selected for biopsy (Parchman and others 1989).

In man and dogs, this is a very sensitive technique for the evaluation of skeletal metastatic disease (Galasko 1987, Lamb 1991). As 50% destruction of medullary bone has to occur in man before a lesion will be visualised radiographically, the uptake of radioisotope can be useful way of demonstrating destruction before it can be detected radiographically (Galasko 1987). Scintigraphy has been further refined by the use of radio-labelled monoclonal antibodies (Epenetos 1987). These have not, as far as the author is aware, been applied to the investigation of the canine disease.

BIOPSY

It is always a worry that biopsy will liberate tumour cells resulting in both local and metastatic spread (Robertson and others 1984). However, the risk of incorrect diagnosis is greater so that the current recommendation (in man) is for biopsy and histological diagnosis (Coombs and Halliday, 1987).

Obtaining a diagnostic sample is the greatest challenge in biopsy of bone tumours. Technically there are a number of problems (see below):

- Lesion may be difficult to visualise without extensive procedure.
- Tumours tend to be heterogeneous.
- Great variation in tissue density through tumour. This may make loosening of a "core" difficult.
- Local inflammatory reaction often intense and difficult to differentiate grossly. A false negative may result from biopsy of inappropriate material.
- Danger of pathological fracture with extensive biopsy.

Current practice in veterinary and human surgery is the used of closed biopsy, though open biopsy is still advocated by some authors (Smith and Sutton 1988). Currently, at GUVS, material is obtained with a Jamshidi bone biopsy needle (Figure 3, page 29) through a minimal surgical approach. These instruments are available in a number of needle gauges and selection depends on patient size and lesion position. Currently 8 and 11 gauge needles are in use in the Department. In potential resection and grafting patients, the approach is directed so that it will be resected during the definitive surgery. This is to reduce the likelihood of leaving a trail of neoplastic cells that may compromise the definitive surgery if limb sparing is undertaken.

The accuracy of diagnosis from biopsy material varies depending on the quantity, quality and area of the sample obtained. Accuracy with the Jamshidi needle has been quoted as 91.9% for the diagnosis of the presence of neoplasia and 82.3% for the identification of tumour type (Powers and others 1988). This compares with a diagnostic accuracy of 93.8% for the Michelle trephine, which takes a larger core of tissue, and is associated with a greater risk of fracture of smaller bones (Wykes and others 1985). Removed tumours are submitted routinely for further evaluation.

There are varying opinions on the best region to biopsy. One study has suggested that the radiographic centre results in more diagnostic information rather than the transitional zone suggested by others (Wykes and others 1985). The taking of multiple samples also might be expected to yield more information and is recommended.

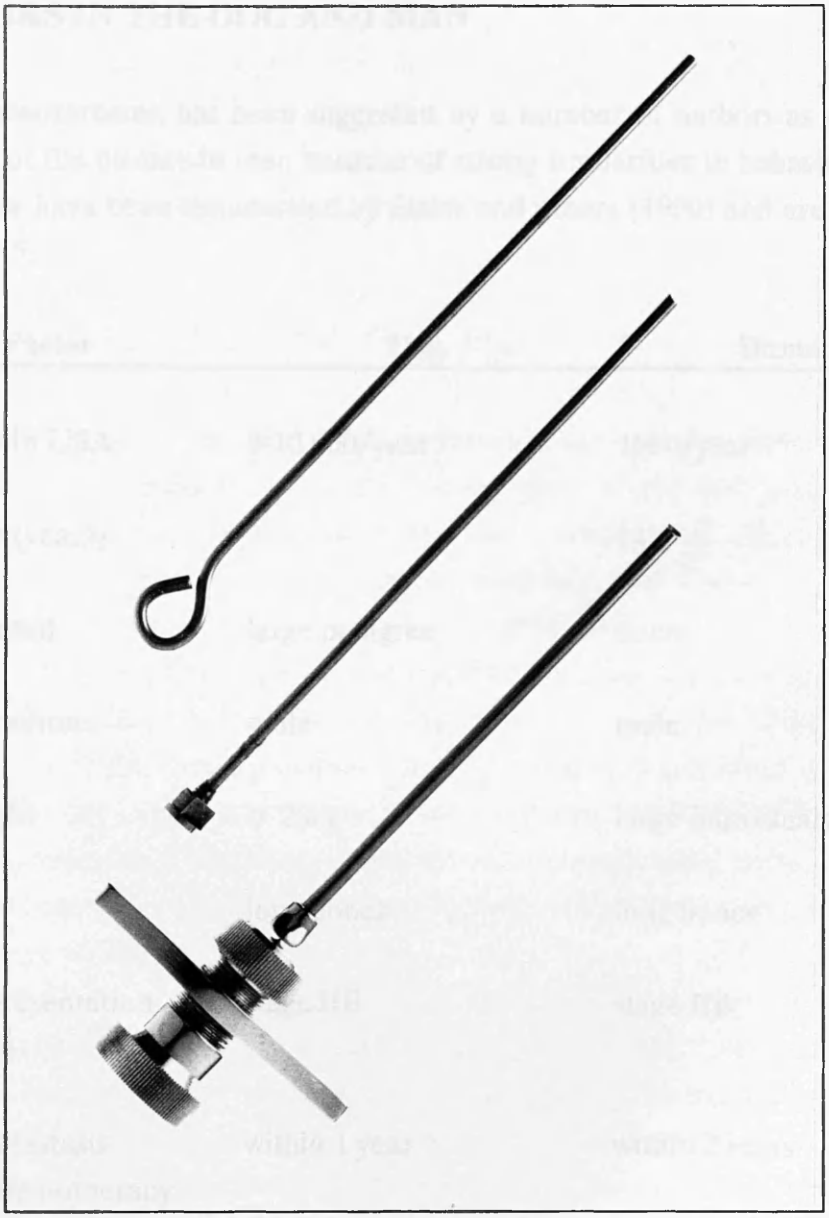


Figure 3. Jamshidi needle

DEVELOPMENT OF MANAGEMENT REGIMES FOR BONE TUMOURS IN THE DOG AND MAN

Osteosarcoma has been suggested by a number of authors as a model for the study of the disease in man because of strong similarities in behaviour. These similarities have been summarised by Straw and others (1990) and are reproduced in Table 15.

Factor	Dog	Human
incidence in USA	8-10,000/year	1000/year
mean age (years)	7	14
race or breed	large pedigree	none
sex distribution	male	male
body weight	> 20kg	large individuals
site	long bones	long bones
stage at presentation (Enneking)	stage IIB	stage IIB
rate of metastasis without chemotherapy	within 1 year	within 2 years
sites of metastasis	lung > bone >soft tissue	lung > bone > soft tissue
effect of chemotherapy	improved survival	improved survival

Table 15. Summary of comparisons between human and canine osteosarcoma (Straw and others 1990).

A number of other common features were identified by Misdorp (1980). These include evidence that fibrosarcomatous osteosarcomas have a generally better prognosis, that tumours appear to occur in stress related areas (if looked at across the species) and that in both man and the dog tumours have been associated with bone infarcts.

There are also differences in the clinical picture. In the dog the disease is seen in mature animals whilst in humans it is primarily, but not exclusively, seen in adolescents. Thus in the dog it is a disease of the mature skeleton whilst in the human it is often observed in skeletons that are still developing. The distribution within the skeleton is different, although all sites are thought to be areas of stress. In man osteosarcoma may follow on from Paget's disease - a disease of bone that is not described in the dog.

MANAGEMENT OF OSTEOSARCOMA

Tumours of the skeleton affect its function as their development results in destruction of the bony structure and compromise of the soft tissue structures along with progressive local discomfort and pain. These local effects are unlikely to be fatal unless they lead to local compromise of a vital structure (this is more likely in the axial skeleton). It is the disseminated metastatic disease that eventually leads to death, usually as the result of euthanasia in the dog.

The classical description of the result of surgical management of osteosarcoma of the limb by amputation in the dog was published as a series of papers by Brodey (with other authors) over the period 1965 to 1975[5]. A number of similar papers have been published that have corroborated these observations (Table 16, page 35). Animals managed by amputation alone can be expected to live for about 18-25 weeks.

Historically it was recognised that the "classic" surgeons response to a neoplasm (i.e. to remove it) had disappointing results when dealing with osteosarcomas in humans. In the 1920's, 80% of osteosarcoma patients managed by amputation died of pulmonary metastasis within a few months (McKenzie 1987). Until the development of effective techniques to manage the metastatic disease, that was how the situation remained. Amputation to relieve discomfort was the only meaningful surgical approach. Surgical management could only be developed once metastasis could be managed to a reasonable degree.

Surgical management of the primary tumour is a technical challenge if anything more than amputation is attempted. In the initial programmes in man, the major complications were related to local recurrence and infection (Grimmer 1987). Experiences with bone allografts have been mixed and currently the use of endoprostheses is advocated by many groups. This is further discussed in Chapters 4 and 5.

[5]Brodey 1965, Brodey and Riser 1969, Brodey and Abt 1976

MANAGEMENT OF METASTATIC DISEASE

It is assumed that pulmonary micrometastases are present at initial evaluation in the majority of patients (90% of dogs managed by amputation alone develop pulmonary metastatic disease within 1 year of surgery). Without adjuvant chemotherapy 80% of human patients with high grade osteosarcoma develop pulmonary metastasis (Westaby 1987). Interestingly, the proportion of people surviving 5 years is very similar to that surviving 2 years. This suggests that survival is related to the extent of metastatic disease at presentation and its control. Individuals with significant metastatic disease or tumours that are insensitive to the chemotherapeutic agents will succumb during this initial period. An ideal therapeutic regime would eliminate these deposits. Currently the best that can be hoped for in both man and the dog is suppression of their development.

Early methodologies were directed at the primary tumour. At the time that radiation therapy was evolving as a therapeutic technique, it was applied to osteosarcomas as a way of trying to control both the primary and the metastatic disease. A variable response was observed and patients with rapidly developing tumours often developed metastasis before surgery was undertaken. Interestingly, some patients were apparently cured following irradiation of the primary and did not require amputation (McKenzie 1987). Further studies in the 50's and 60's reported further limited success in the control of the primary tumour. There seems to be a wide range of response to radiation therapy with occasional tumours being very sensitive. This may relate to an apparent subgroup of potentially radiosensitive small cell osteosarcomas described above (Table 6, page 9).

Application of radiation to the lung field as a method of controlling pulmonary metastasis was subject to randomised trial and showed some increase in survival rate at 5 years (McKenzie 1987). Other studies have failed to substantiate these observations (Westaby 1987). This work was published at a similar time as the reports of chemotherapy that were producing more dramatic results. No beneficial effect was observed following pulmonary irradiation in the dog (Owen and Bostock 1973).

In contrast, radiation therapy of the primary tumour has been demonstrated to be of little value in the canine (Thrall and others 1990). This is probably because the small cell osteosarcomas do not occur in dogs. Radiation therapy, however, has been demonstrated to have a palliative effect in controlling pain though this returns in 4-6 months (McGlennon 1991).

Chemotherapy has been demonstrated to have a positive benefit in the management of human osteosarcomas and has greatly improved the prognosis of what was previously a hopeless cause. Methotrexate, doxorubicin and cisplatin are the currently favoured drugs (Yasko and Lane 1991). Many of the protocols

involve combination chemotherapy with cisplatin and doxorubicin being popular. Extrapolating from this success, similar protocols have been applied to canine patients with varying degrees of success (Table 20, page 68-72). However, it has been fairly conclusively demonstrated that the use of cisplatin increases the disease free interval and life span of selected canine patients. Chemotherapy does not prevent or destroy metastatic tumours.

In the management of human patients with pulmonary metastasis, metastectomy has been shown to be effective in selected patients (Westaby 1987). Resection of solitary pulmonary nodules as a method of controlling limited metastatic disease in the dog is described (Straw and others 1990). The general impact on survival in the dog is unknown due to the small number of patients. In one group of 22, the median survival time post thoracotomy was 3 months. One dog was still alive 36 months following metastectomy.

Immunomodulation using BCG[6] has been attempted in the dog (Owen and Bostock 1974). BCG stimulates a non-specific infiltration of the lung parenchyma with macrophages and "natural killer cells". These are associated with the control of neoplastic cells and a local increase in numbers ought to reduce the numbers of metastatic cells. Though positive effects were demonstrated in a small number of animals there were problems with anaphylaxis and repeated injections are required to maintain the effect. Two dogs with apparently early tumours were managed using this protocol at Bristol Veterinary School both of which were euthanased because of skeletal metastasis within 6 months (Gibbs and others 1984).

Another immunomodulation technique utilising muramyl peptides has been described (McEwan 1990). These again are non-specific stimulants of the immune system, increasing antibody production and encouraging cellular immunity to an antigen. In a double blind trial significant increases in both disease free interval and life span were observed. Patients tolerated therapy well.

EVIDENCE THAT NOT ALL OSTEOSARCOMAS HAVE THE SAME BEHAVIOUR

A number of features of the biological behaviour of canine osteosarcoma suggests that there are biological subsets of the tumour. However, the range of behaviour reported does not appear to be as diverse in the dog as it is in man. There are rare but consistent reports of animals surviving for long periods with known tumours and no evidence of metastasis (Brodey and Abt 1976). Likewise there are consistent but infrequent reports of apparent cure by amputation (Brodey 1965, Brodey and Abt 1976, Spodnick and others 1991).

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[6]Bacillus Calmette Guerin

Osteosarcomas in certain areas of the skeleton differ in prognosis. In particular, those of the skull have a comparatively long interval to metastasis whilst those of the hind limb have been associated with aggressive behaviour by one author (Misdorp 1980) whereas contrary observations have been reported by another (Brodey 1965). Amongst tumours treated with chemotherapy, there is a noticeable variation in the response with occasional instances in which there are strikingly long periods of remission. Radiographically atypical lesions have been associated with apparently different behaviour (Gibbs and others 1984).

SUMMARY

Osteosarcomas are relatively uncommon tumours in the dog (though the incidence is much higher than in the other domestic species and man). They are of uncertain aetiology though there a number of well-recognised risk factors. Other sarcomas of the skeleton of the dog are rare (in contrast to man but in keeping with the other domestic species). Benign bone tumours are very rare in dogs.

The cell lineage of the varies sarcomas of bone is unknown. In the dog they are primarily osteosarcomas However, at what point along the differentiation pathway does transformation occur ? Whether the various sarcomas are of the same origin or represent neoplastic transformation of different lineages is uncertain.. It is likely, however, that the transformed cell type is associated fairly specifically, at some stage, with osseous tissue as extraskeletal bone and cartilage tumours are exceedingly rare in both man and the dog (Patanik 1990). Some pointers to the origin of the affected cell types comes perhaps from the analysis of sarcomas that occur following fractures. Primitive cell types related to bony development, perhaps trapped in the bony structure, undergoing malignant transformation are a possible origin. This may also be relevant to the metaphyseal distribution.

The commonest site of osteosarcoma development is the appendicular skeleton. Tumours of the axial skeleton show a much higher incidence of chondrosarcoma, which is generally less aggressive. The osteosarcomas that develop in this region are also reputed to have lower rates of metastasis.

It is known that the flat bones and the long bones have essentially different patterns of development. It could be speculated that the type of bone influences the type of sarcoma that are likely to develop. It is also possible that the type of bone and anatomical region in which a sarcoma develops influence its behaviour.

Author	Date	Number of patients	Median survival (weeks)	One year survival	Disease free interval (weeks)
Brodey	1965	31	NA	4/29 (14%)	NA
Brodey and Riser	1969	41	NA	6/41 (15%) **8 months survival**	NA
Brodey and Abt	1976	65	18 (range 3-578)	7/65 (10.7%)	NA
Mauldin and others	1988	19	25 (range 3.2-68.6)	4/19 (21%)	22.8 (pertains to 9/10 patients)
Shapiro and others	1988a	8	14.5 (range 8-46)	NA (no survivors)	13-33
McEwan	1990	13	11	2/13 (15%)	8.3 (4.4-32.4)
Spodnick and others	1991*	162	19.2 (range 3-214)	11.5%	NA

Table 16. Summary of reported survival times for the management of appendicular osteogenic sarcoma by amputation alone.

*In press

Prognosis is thought by some authors to vary between the various classes of osteosarcoma identified. This seems to be a subjective opinion in that their low incidence makes this difficult to demonstrate statistically. The more fibrous tumours are thought to have the best prognosis, in a similar fashion to man (Misdorp 1980), whilst the telangiectatic ones are thought to have the bleakest outcome, again similar to man (Gleiser and others 1981).

Osteosarcomas are classified into histological groups, though this does not reflect their potential behaviour. A number of staging systems are in use in the human field but none yet has been widely accepted in veterinary work. This is of particular relevance when comparisons of the results of therapy are made not only within groups of reported animals but also between centres under taking similar and differing modes of management. It would be useful if a staging system was adopted as standard to facilitate the assessment of the management regimes.

Amputation is thought of as being palliative with respect to the development of metastatic disease in both man and the dog. However, there is probably an increase in lifespan of selected patients as amputation gives excellent control of associated pain and hence is likely to delay the time to euthanasia (euthanasia due to chronic pain being likely to occur before that due to clinically significant metastatic disease).

Though the results of chemotherapeutic management of the disease in the dog have perhaps not been as encouraging as in man, there have been significant improvements in life expectancy associated with the use of cisplatin, in conjunction with both amputation and limb salvage procedures.

CHAPTER 2.

PLATINUM CONTAINING ANTI-NEOPLASTIC AGENTS: DISCOVERY AND OVERVIEW OF USE IN HUMAN ONCOLOGICAL PRACTICE

DISCOVERY AND DEVELOPMENT OF CISPLATIN

The initial observation that led to the development of cisplatin was made during a series of experiments to assess the growth characteristics of *Escherichia coli* in electromagnetic fields (Rosenberg and others 1965). The use of platinum electrodes inhibited cell division and the bacteria developed as long, filamentous forms. This was demonstrated to be due to the production of electrolysis products, formed because of the presence of ammonium chloride in the growth medium. These were principally *cis*-diamminetetrachloroplatinum (IV) and *cis*-diamminedichloroplatinum (II).

The development of these filamentous forms of the bacteria implied, in the presence of otherwise unaffected cell functions, interference with DNA replication. A similar effect is observed with other DNA affecting agents such as ultraviolet light, high energy radiation and alkylating agents (Pinto and Lippard 1985).

This observation was pursued and a group of platinum containing complexes that inhibited cell activities was isolated. The more ionised complexes tended to be bactericidal whilst the neutral ones had an inhibitory effect on division. The stereo-chemistry of the complexes was demonstrated to be significant with the complementary *trans* form, though having some bactericidal activity, having no effect on cell division (Pinto and Lippard 1985).

The intuitive step was to test these compounds against established mouse tumour models (Rosenberg 1985). Success in the animal model led to clinical trials on terminal human patients, with encouraging results. As a result of these observations and many other experiments, cisplatin was licensed for clinical trials in 1972, with full Federal Drug Authority approval following in 1979. It is now a major part of the armamentarium in human oncological chemotherapy (Pinto and Lippard 1985).

USE IN THE HUMAN PATIENT

Cisplatin has been assessed against many human malignancies and has found its most frequent applications in the management of soft tissue neoplasia (below).

testicular
metastatic ovarian
cervical
lung
bladder

It has also used in the management of osteosarcoma in combination with doxorubicin.

In human oncology cisplatin is of major importance as a chemotherapeutic agent. It has had a marked impact, in combination with vinblastine and bleomycin, on the management of testicular tumours, a major cause of non-traumatic mortality in young men, in which the cure rate is now approximately 70% (Loehrer and others 1991). The drug also is of importance in the management of ovarian tumours and inoperable solid sarcomas.

The development of chemotherapy regimes in general has had an impact on survival times for patients with osteosarcoma. Long term survival (greater than 5 years) has been reported to be 50-76% (Yasko and Lane 1991).

TOXICITY

Initial clinical application of cisplatin in humans quickly confirmed the renal toxicity that had been observed in experimental animals (Rosenberg 1985). In addition, other toxic effects became apparent. Toxic effects are correlated with both the magnitude of plasma levels achieved during infusion and with duration of exposure to the drug. There are a number of clinically important toxic effects induced by cisplatin in human patients (Table 17).

Toxic effect	Mechanism
renal tubular necrosis	major damage in the distal part of the proximal tubule (cf the dog, page 50)
electrolyte wasting	particularly magnesium due to interference with uptake
bone marrow suppression	depression of myeloid and platelet series
peripheral neuropathies including loss of high level acuity in hearing	damage to primary sensory neurones in the spinal ganglia (Cavaletti and others 1991) overdose may produce acute central nervous toxicity (Fassoulaki and others 1989)
acute gastrointestinal toxicity	damage to the rapidly dividing cells of the gastrointestinal mucosa
nausea and vomiting associated with infusion	centrally mediated can be severe enough to prevent patients returning for further cycles of their treatment
ototoxicity	damage to the outer hair cells of the cochlea (Gandara and others 1991)

Table 17. Toxic effects of cisplatin in man.

The renal toxicity of cisplatin is broadly proportional to the dose given. Assessment of renal function is important and the dose administered may be altered depending on an individuals renal function. In a group of patients undergoing multiple cycles of cisplatin, those ultimately developing renal toxicity exhibited significantly higher levels of plasma platinum during the infusion (Campbell and others 1983). Interestingly, in these patients pre-treatment creatinine levels did not correlate with renal toxicity (however, a more useful parameter would have been measurement of renal function - see below).

The development of peripheral neuropathy is a feature of repeated cycles of the drug (Gandara and others 1989). The administration protocol has been implicated in the development of neuropathies in man and experimental follow up in a rat model has shown differences in platinum concentration in nervous tissue depending on protocol but with the same total dose (Cavaletti and others 1991).

Cisplatin affects the stem cells related to the production of granulocytes and platelets. The factors that influence the renal toxicity are not well correlated with the development of myelosuppression.

RENAL TOXICITY

The cellular mechanism of cisplatin toxicity is unknown (Daugaard and Abidgaard 1989). There are problems interpreting the pathophysiology of the renal insult because it appears to differ between species. There is also variation in the response between different areas of the kidney. A heavy metal type of toxicosis related to activation of the renin-angiotensin system was thought to be a component of the toxic effect (Shapiro 1989), though this is not now thought to be the case (Daugaard and Abidgaard 1989).

The major region of renal insult is the proximal convoluted tubule (Daugaard and Abidgaard 1989). Cisplatin toxicity is initiated by an acute tubular impairment followed by changes in renal blood flow. At 48-72 hours post administration there is impairment of both proximal and distal tubular function and an increase in vascular resistance (Daugaard and Abidgaard 1989). The nephrotoxic action is thought to be related to the main aquation product of cisplatin (Daugaard and Abidgaard 1989). This may be one of the reasons carboplatin (another platinum containing chemotherapeutic) is less nephrotoxic (Daley-Yates 1985).

Parameters such as plasma creatinine and urea are not good indicators of renal function. They are only become altered in uncompensated renal disease and as such are of little use in detecting subtle effects on renal function. They are also affected by the state of other body systems (e.g., liver dysfunction will affect urea production; cachexia will affect creatinine release).

Previously, the preferred method of assessing glomerular filtration rate was endogenous creatinine clearance as it is relatively non-invasive. However, because of problems with the technique it has now been replaced by ^{51}Cr-EDTA clearance (Daugaard and Abildgaard 1989).

AMELIORATION OF TOXICITY

It was quickly discovered that toxic side effects could be ameliorated by influencing renal function and manipulating the time over which the drug was given (Rosenberg 1985). It is now routine clinical practice to administer cisplatin in association with pre-infusion fluid loading and diuretics.

The action of the diuretics that is related to renal protection is uncertain (Safirstein and others 1985, Daugaard and Abildgaard 1989). However, it is thought that the partial renal protection offered by these drugs in humans is not attributable to changes in urinary excretion, plasma clearance or reduced levels of platinum (Daugaard and Abildgaard 1989). The use of frusemide has not been shown to have a positive benefit in humans and is not recommended (Daugaard and Abildgaard 1989).

It is known that in the initial phase platinum is excreted rapidly by the kidneys. This is obviously increased by diuresis but this does not seem to adversely affect the therapeutic effect (Hayes and others 1977). It has also been demonstrated that giving the drug as a prolonged infusion decreases toxic effects without reducing therapeutic efficacy (Hayes and others 1977).

The use of hypertonic saline has been demonstrated to have a protective effect on the kidneys. Studies on platinum in the plasma ultrafiltrate suggest that a protocol involving saline pre-hydration and a hypertonic saline vehicle for the cisplatin encourage the uptake of the drug by the tissues of the body (Dumas and others 1990). The use of this combination resulted in significant decreases in the maximal plasma free platinum concentration, the degree of protein binding after infusion and reduced urinary excretion.

Toxicity is also a function of the time of day the drug is administered. These effects have been observed in humans (Hecquet and others 1985), dogs and rats (Hardie and others 1991). This relationship to the circadian rhythm may be related partly to the diurnal variation in plasma proteins but it must be remembered that renal function increases at a similar period (Hecquet and others 1985). Diuresis has been shown to be most effective at protecting rats when applied during the most favourable period of the circadian cycle.

Though the development of less toxic platinum containing drugs is one method to approach the side effects, these have so far also had significant toxic effects. The concepts of protection and rescue are well recognised in

chemotherapy and are used routinely with some drugs (e.g., methotrexate and folic acid). The use of saline diuresis and mannitol diuresis have been shown to protect against nephrotoxicity. A number of drugs have been developed that protect cells from the effects of cisplatin.

WR-2721 is an organic thiosulphate that, after biotransformation, enters cells and chelates cisplatin metabolites, inactivating them. Differences in pH and alkaline phosphatase between normal and malignant tissues result in a higher uptake in normal cells. Clinical trials demonstrated protection against nephro-, oto-, neuro- and myelo- toxicosis whilst antitumour activity was maintained (Gandara and others 1991).

Diethyldithiocarbamate (DDTC) is a chelating compound with an established clinical use for nickel and cadmium poisoning. The protective effect is by chelation and removal of tissue bound platinum. The DNA adducts that are responsible for cisplatins antitumour effect are not affected. Protection against nephro-, gastro- and myelo- suppression have been demonstrated in animal models. DDTC is unique in that of all the proposed protecting agents it can be given effectively both before and after cisplatin therapy (Gandara and others 1991).

A number of potentially useful drugs are under evaluation. It can be seen that any single drug does not protect against all the potential toxic effects of cisplatin. For example thiosulphate, though protecting against nephrotoxicity, does not protect against gastro- and myelotoxicity in the way that DDTC does (Leeuwenkamp and others 1990).

Myelosuppression can be ameliorated by the use of recombinant granulocyte colony stimulating factor (Saito and others 1990).

ADMINISTRATION

There are a number of administration protocols described for cisplatin in human oncology. Authors state that the ideal technique has yet to be described. Doses can be very high with protocols involving doses of 180-220 mg m^{-2} described. The standard dose is in the region of 50-70 mg m^{-2} every 3-4 weeks (Gandara and others 1989).

Cisplatin is administered intravenously, intra-arterially and intraperitoneally depending on tumour type and location. In the therapy of osteosarcoma where limb salvage is envisaged, it is popular to give an intra-arterial infusion. This has led to an increase in the number of limb salvage procedures, as it reduces the bulk of the tumour. To date, there has been no demonstrable benefit to long term survival using this technique as opposed to intravenous therapy (Yasko and Lane 1991).

PHARMACOKINETICS

The parent drug has a short $T_{1/2}$ in the plasma of about 30 minutes, with 40% of the dose appearing in the urine within 2 hours (Reece and others 1987). The decay of platinum in the plasma ultrafiltrate has been reported by a number of authors and is presented in Table 18. It is described as being uni- or bi-exponential and is fairly rapid. It is thought that after 4 hours the platinum in the plasma ultrafiltrate represents metabolites and products of aquation of uncertain cytotoxicity (Reece and others 1987).

Author	Dose (mg m^{-2})	Duration of infusion (minutes)	$t_{1/2}\alpha$ (minutes)	$t_{1/2}\beta$ (minutes)	Exponentials
Gullo and others (1980)	50	60	22		1
Belliveau and others (1976)	125	7200	178		1
	120	30	39		
Reece and others (1987)	80	120	35.6		1
	100		27.4		
	120		29.0		
Erlichman and others (1987)1	50	15	1.7	46.2	2
Dumas and others (1990)	100	20	22.8		1
Saito and others (1990)	25	4200	not stated	126	2

Table 18. Decay of platinum in plasma ultrafiltrate in human studies, following infusion.

The area under the concentration-time curve (AUC) for platinum in plasma ultrafiltrate given as a 5 day continuous infusion of 125mg m^{-2} (in total) was 0.16 ng min l^{-1} (Belliveau and others 1976) and 0.15 ng min l^{-1} (Saito and others 1990). A thirty minute infusion of 120 mg m^{-2} had an AUC of 0.08 ng min l^{-1} (Belliveau and others 1976). Urinary excretion is reduced by prolonged infusions, suggesting increased retention by body tissues.

[1]The original paper is confusing on this data and this is the author's interpretation.

OTHER PLATINUM CONTAINING CHEMOTHERAPEUTICS

The development of cisplatin quickly established that renal toxicity was a significant dose limiting factor. This stimulated the search for other platinum containing compounds with less toxicity.

An analogue of cisplatin - carboplatin (diammine 1,1-cyclobutanedicarboxylate platinum II) is currently licensed for use in man (Paraplatin, Bristol-Meyers Pharmaceuticals[2]). Like cisplatin, carboplatin undergoes activation by aquation. It is known that one of the metabolites of carboplatin is cisplatin (Daley-Yates 1985).

Carboplatin does not exhibit the dose limiting renal toxicity of cisplatin. This obviates the need for the complex hydration protocols. It does, however, have significant toxic effects on the bone marrow that produce dose limiting effects (Calvert and others 1989) and has also been shown to be ototoxic, though clinically significant deafness does not occur under normal usage (Kennedy and others 1990). These effects are given as a reason that carboplatin is unlikely to replace cisplatin in routine clinical work (Gandara and others 1991).

Carboplatin is currently recommended for ovarian carcinoma and small cell carcinoma of the lung. Clinically it has similar anti-tumour activity to cisplatin but has enhanced activity against human small cell carcinomas of the lung. Not all tumour lines show cross resistance with cisplatin (Daley-Yates 1985). These observations suggest some difference in mechanism.

OTHER ANALOGUES UNDER DEVELOPMENT

The success of platinum containing drugs in chemotherapy has provided the commercial stimulus for the further investigation of analogues of cisplatin to find the ideal drug with high efficacy and low toxicity. Carboplatin is licensed and iproplatin and (glycolate-0,0') diammineplatinum (II) are under evaluation (Fukuda and others 1990).

Another group of compounds selected for further investigation are organic dye - platinum complexes. These are hoped to have the membrane permeability of the dye component and the cytotoxicity of the platinum containing drugs (Page, personal communication[3]).

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[2]Bristol-Meyers Pharmaceuticals, Uxbridge, UB10 8NS

[3]Dr. R. Page, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, 27606.

SUMMARY

Cisplatin is the first of a family of platinum containing chemotherapeutic agents. The discovery of this group is an interesting example of serendipity, though the application of Rosenberg's observations to models of cancer was an intuitive step.

Though cisplatin has revolutionised the therapy of certain sarcomas in man it is not without significant morbidity from associated side effects. As the response is dose dependant, these limit the potential anti-tumour activity. Hence the amount of effort devoted to improving efficacy whilst reducing side effects and the search for new less toxic analogues. Though nephrotoxicity was the first significant problem to be encountered, this is now well managed and the most important dose-limiting toxicity is in the form of peripheral neuropathies which can confine patients to a wheelchair (Gandara and others 1991). Along with the development of new platinum drugs goes the search for protective drugs that can be administered in conjunction with cisplatin to reduce its side effects.

CHAPTER 3.

PHARMACOLOGY OF CISPLATIN IN THE DOG

USE OF CISPLATIN IN THE CANINE PATIENT

Though cisplatin was licensed for use in humans in 1979 and dogs had been a major experimental animal during the initial pharmacological evaluation, the first reports of its therapeutic use in animals only appeared in the mid 1980's (Melhaff and others 1984, Page and others 1985). The first publications were anecdotal reports of the drug's use in a number of clinical situations (Table 20, pages 68-72). Initial experience demonstrated some beneficial effect rather than the spectacular results that were so exciting in some of the human tumours. However, sufficient effect was observed to encourage further investigation and to focus on the protocol for administration that should be adopted.

Initially little clinical work was performed due to the prohibitive cost of the drug and the technical problems associated with its administration (Page and others 1985). Many of the later papers acknowledge the gift of the drug by the manufacturers. Currently in the USA, clinical use is declining because of the less readily available supply of "free" drug and client resistance to the full economic cost (R. Page, personal communication[1]). One of the biggest institutions reporting on the clinical use of the drug (Colorado State University) is readily given osteosarcoma referrals from other veterinary institutions because of their current ability to defray costs of therapy due a number of funded projects. The cost of drugs for these patients was not passed on to owners.

PHARMACOKINETICS

Most of the work on the fate of cisplatin in the dog has looked at total plasma platinum and tissue distribution. Some aspects of the fate of platinum in the plasma ultrafiltrate are described (Riviere and others 1990, Hardie and others 1991). It was appreciated in the early experiments that there was a biphasic decay of plasma platinum with a rapid early phase ($t_{1/2}\alpha$ of <1 hour) and a prolonged terminal half life ($t_{1/2}$ β of 4-5 days). Platinum was still detectable in plasma 12 days after administration (even by relatively insensitive analysis). Within 4 hours 50-60% of the administered platinum is excreted in the urine (Litterst and others 1976).

After intravenous administration, the retention by different organs is not uniform. The greatest concentrations are seen in the kidneys. The parenchymatous organs such as liver, uterus and lung have similar concentrations, about 30% of that seen in the kidney tissue (Litterst and others 1976). The

[1]Dr. R. Page, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, 27606.

relative distributions are not altered by the dose administered but can be altered by whole body hyperthermia (Reviere and others 1990).

Cisplatin is an inorganic co-ordination complex with four ligands. These ligands are the reactive parts of the complex and the drug's activity relates to their substitution, which depends on the environment in which the compound is present. The two chloride groups are the most reactive and undergo hydroxylation in an aqueous environment. The rate of this reaction is related to the chloride concentration. In the high chloride environment of the extracellular fluids of the body (103mM) this equilibrium does not proceed to any extent (Daley-Yates 1985). In the low chloride cellular environment (3-4mM) the production of the highly reactive substituted species is encouraged (Lim and Martin 1976, quoted by Daley-Yates 1985). A number of platinum species are produced, the distribution being related to properties of the environment. It has been estimated that in the plasma of mammals the various hydrolysed species represent about 2% of the total platinum (Lim and Martin 1976 quoted by Daley-Yates 1985) whilst in the intracellular environment it is approximately 42% (Lim and Martin 1976 quoted by Pinto and Lippard 1985).

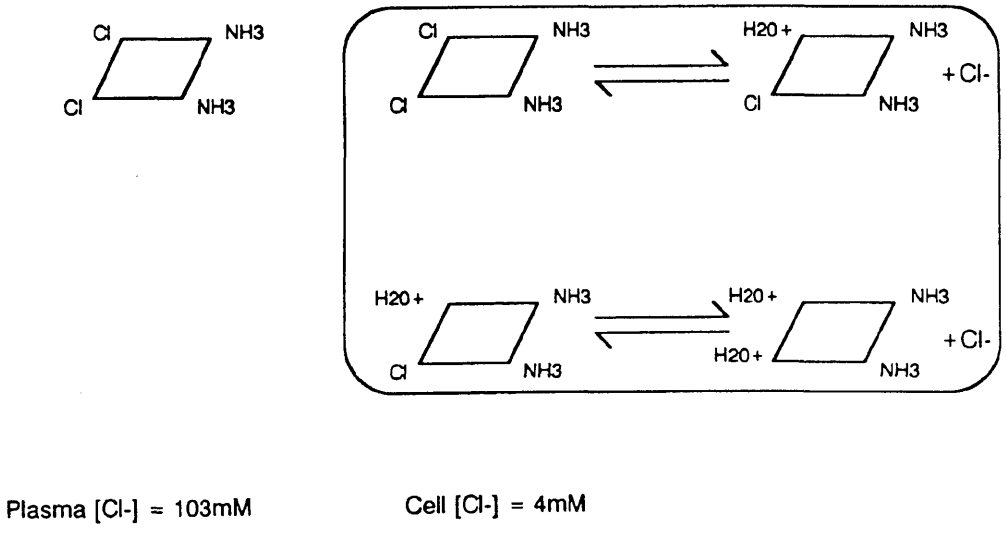

Figure 4. Aquation of cisplatin[2].

WHAT IS IT IN THE CIRCULATION THAT IS RESPONSIBLE FOR THE ACTIVITY

Cisplatin is an unreactive compound that undergoes passive activation (aquation) by hydrolysis of the chloride ligands (Siddik and others 1985). The

[2]Adapted from Gandara and others 1989

parent drug enters the cell by passive diffusion (Rosenberg 1985). The active form(s) are a product of aquation. There may be a number of platinum species generated that are related to the clinical action. Daley-Yates (1985) suggest that the nephrotoxicity of cisplatin is related to the amount of its primary toxic metabolite that develops in the plasma.

CELLULAR ACTIVITY

Cisplatin induces its cytotoxic effect by binding to DNA. Replication is halted but the continued growth observed *in vitro* suggests that RNA and protein synthesis are essentially unaffected (Pinto and Lippard 1985). The effect is on DNA as template rather than on the enzymatic processes that are involved in DNA synthesis. The failure of the DNA template is related to the inter- and intrastrand cross linkages that form between the cisplatin and DNA. It is likely that the interstrand cross linkages are of limited importance in the biological activity of cisplatin, with most importance being attached to the intrastrand linkages (Pinto and Lippard 1985). There are also linkages formed between cell proteins and DNA though the significance is uncertain. Cisplatin affects cells during all phases of the cell cycle but the effect is realised during cell division (Shapiro 1989).

There is evidence that one mechanism of cisplatin resistance is related to the cellular DNA repair processes. Cell lines with known deficiency in this process are more susceptible to the cytotoxic effects of cisplatin (Pinto and Lippard 1985).

THE REASONS FOR ASSESSING UNBOUND PLATINUM IN THE PLASMA

Cisplatin is almost irreversibly bound to plasma proteins, in particular albumin (Briand and others 1986). Some protein-bound platinum will react with strong nucleophiles, though this is probably only of relevance in rescue procedures that utilise such compounds (Hegedus and others 1987). It also binds to other constituents of plasma, not all of which are identified. Though there is some binding to cells in the circulation, this is a small proportion (see page 58). There is also irreversible binding to tissue proteins. Though some of this may be released at low levels over prolonged periods, the amounts involved are unknown and interest is in the relation to toxicity rather than clinical effect (Matheson and others 1989). Platinum will also be released by the turnover of plasma proteins (King and others 1986).

It is thought that the pharmacologically active fraction of the drug is that which is unbound in the plasma (Hardie and others 1991). This is usually assessed by measuring platinum levels in plasma ultrafiltrate. However, as the molecular

weights of all plasma constituents to which the drug binds is not known, there is no consensus on what size of filter to use. Riviere and others (1990) demonstrated no difference in apparently unbound platinum when using a variety of filter sizes (10-30,000 Daltons). It must be remembered that the platinum concentration does not represent levels of cisplatin *per se* but will include its aquation products as well (Reece and others 1987).

TOXICITY

Most of the toxic effects seen in man are encountered in the dog with the exception of the peripheral neuropathies and ototoxicity (transient incidence of LMN disease reported in one dog, Melhalff and others 1984). In man these are problems of repeated cycles of the drug, which are not routinely utilised in the dog. A single case of myasthenia has been reported associated with cisplatin chemotherapy (see page 11) (Moore and others 1990). One instance of grand mal seizures and sudden death 3 hours after administration has been observed (Knapp and others 1988). The major side effects observed in the clinical application of the drug in the dog are summarised in Table 19 (pages 52-57). Experience with cisplatin has shown it to be of tolerable toxicity in the dog if carefully administered and the patients monitored. Close monitoring does reveal some sub-clinical toxic effects.

Renal toxicity has not been a major clinical problem though in those studies where it was most meaningfully assessed (using creatinine clearance) there was evidence of progressive depression in renal function in some individuals (Shapiro and others 1988a, Shapiro and others 1988b). Sporadic instances of individual animals developing azotaemia occur throughout the literature. However, the design of administration protocols has led to amelioration of the clinical problem.

The major area within the kidney affected by cisplatin is the s3 segment of the proximal convoluted tubule (Daugaard and Abildgaard 1989). The immediate effect of cisplatin administration in the dog is a decrease in the fractional and absolute proximal resorption rates of sodium and water. This occurs despite the renal blood flow and glomerular filtration rate remaining unchanged during the initial period. In the 48-72 hour period there is a dysfunction in the distal tubule resorption capabilities and a rise in vascular resistance. There is polyuria as a result of impairment of the renal concentration mechanism (Daugaard and Abildgaard 1989). Some of the renal toxicity was previously attributed to a heavy metal influence on the renin-angiotensin system (Shapiro 1989) but this is now thought to be unlikely (Daugaard and Abildgaard 1989). The cellular mechanism of renal toxicity is unknown (Daugaard and Abildgaard 1989).

The toxicity of cisplatin is time dependant in several species. There is significantly greater toxicity in the afternoon as compared to the morning. This is related to significant changes in the pharmacokinetics of drug. Afternoon administration is associated with significant decrease in the mean residence time of the platinum in the plasma ultrafiltrate and a significant increase in urinary excretion of platinum. There was a trend for an increase in clearance coupled with a decrease in the area under the time concentration curve and volume of distribution at a steady state but these were not significant (Hardie and others 1991).

In more recent clinical reports, myelosuppression has been the most prevalent toxic effect. The dog model used in experiments to confirm the usefulness of mannitol diuresis in protecting against renal toxicity showed no protection from myelosuppression (Cvitkovic and others 1977).

Vomiting after therapy has been described by many authors though in no instance has it been cited to be dose limiting. The associated nausea that is so well recognised in humans is impossible to assess objectively in the canine patient and may be underestimated. Most protocols make some attempt to ameliorate this potential side effect using drugs such as metoclopramide and chlorpromazine. In a retrospective study of 115 cases, the total dose of cisplatin was identified as the most significant factor in the incidence of emesis (Ogilvie and others 1989a). It was significantly more prevalent in smaller dogs, which may be related to the proportionally higher doses these animals received (doses are based on surface area) and possibly poor assumptions used in the construction of the tables for calculating surface area from body weight. Disappointingly, pretreatment with anti-emetics did not reduce the incidence of vomiting.

Author	Date	Dose	Administration protocol	Monitoring	Toxic effects noted
Melhaff and others	1984	60mg m^2 cisplatin on days 1 and 29 of a 42 day protocol given in combination with vindesine (0.1mg/kg)	saline diuresis for 12 hours pre-infusion (rate not stated). cisplatin infused over approximately 1 hour post-infusion saline diuresis maintained for 18 hours at pre-infusion level mannitol (2mg/kg) i/v for 20 minutes immediately before cisplatin	assessment protocol not stated	no azotaemic episodes noted mild leucopaenia which resolved transient rear limb weakness with reduced patellar reflex ? vomiting stated that was controlled
Himsel and others	1986	initially 25mg/m^2 (1) but raised to 60mg/m^2 (2) given for one dose after apparent remission. No clinical effect until given at higher dose. second case 60mg/m^2 administered 3 weekly to effect	diuresis with 0.9% saline for 3 hours pre-infusion at 25ml/kg/hr. cisplatin administered over 20 minutes possibly separately post infusion fluids given at 12ml/kg/hr for 3 hours mannitol (18.75g/l saline) added to saline and continued through infusion and post infusion. chlorpromazine 0.5mg/kg i/v if vomiting occurred	BUN, WBC, PCV, and platelet values were assessed immediately before and daily for 10 days post therapy	1) after 8 cycles of increasing cisplatin dose demonstrated a rise in BUN which persisted for 6 months. 2) individual demonstrated no change in BUN 1) mild leucopaenia following each cycle (not stated whether dose dependent) no change in platelets 2) depression of platelet values but no change in leukocyte count 1) some administrations induced vomiting 2) all administrations caused vomiting

Table 19. Reported use of cisplatin in the dog and toxic side effects

Author	Date	Dose	Administration protocol	Monitoring	Toxic effects noted
Mauldin and others	1988	60mg/m^2 in combination with doxorubicin @30mg/m^2 given as two courses over 8 weeks (cumulative dose 60mg/m^2 doxorubicin and 120mg/m^2 cisplatin)	diuresis 30ml/kg 8 hours pre and 8 hours post cisplatin given over 8 hours mannitol @ 500mg/kg i/v dexamethasone @ 0.025mg/kg i/v prochlorpromrazine 0.1mg/kg i/v	BUN, WBC, RBC, creatinine, platelet count, urine analysis before each administration	haematological parameters and urinalysis remained unchanged incidence of vomiting not reported
Shapiro and others	1988a	50mg/m^2 40mg/m^2 if creatinine clearance <1 standard deviation below the mean 2-6 courses. evidence of metastatic disease no further treatment	diuresis of saline 12-18 hours before infusion at 105ml/m^2/hr 6 hours post infusion at the same rate mannitol administered before infusion (15mg/m^2 in 70ml saline/m^2/hr) metoclopramide given to some dogs	CBC, BUN, creatinine, serum electrolytes, urinalysis, 6 hour endogenous creatinine clearance before each administration	1/11 dogs developed any haematological abnormality which was a platelet level of $187x10^{12}l^{-1}$ after 3 cycles BUN, creatinine and electrolytes remained within normal limits gradual decreases in creatinine clearance were observed in 3/11 dogs vomiting was observed in 9/11 dogs no animal vomited >6 hours after administration

Table 19 (cont). Reported use of cisplatin in the dog and toxic side effects

Author	Date	Dose	Administration protocol	Monitoring	Toxic effects noted
Shapiro and others	1988b	50mg/m^2 over 6-8 hours at maintenance in saline (70ml/m2/hr) 40mg/m^2 if creatinine clearance < 1 standard deviation below the mean 2-6 courses. evidence of metastatic disease no further treatment	diuresis of saline 12-18 hours before infusion at 105ml/m^2/hr 6 hours post infusion at the same rate mannitol administered before infusion (15mg/m^2 in 70ml saline/m^2/hr) metoclopramide given to some dogs	CBC, serum biochemistry, urinalysis, endogenous creatinine clearance sodium sulfinalate clearance (if urethral catheterisation not possible) were performed routinely before each monthly cycle	thrombocytopaenia was detected in 2/13 dogs: 1) 2 weeks post administration 185x10^9l^{-1} normal by next cycle 2) 10 days post administration 51x10^9l^{-1}, increasing to normal within a week 9/13 dogs showed evidence of suppressed renal function following cisplatin administration vomiting was observed in 7/13 dogs no animal vomited .6 hours after administration

Table 19 (cont). Reported use of cisplatin in the dog and toxic side effects

Author	Date	Dose	Administration protocol	Monitoring	Toxic effects noted
Knapp and others	1988	$60mg/m^2$ variable number of courses depending on response: PR 3 weekly until relapse CR 3 weekly for 2 cycles after remission	diuresis 20ml/kg/hr 4 hours before and 2 hours after 0.9% saline 20 minute infusion in 0.9% saline no anti-emetic unless vomiting occurred no diuretic	CBC, platelet counts, SUN, creatinine, before each cycle CBC and platelet count at 10 days post infusion	thrombocytopaenia with granulocytopaenia occurred in 2/41 thrombocytopaenia only in 2/41 granulocytopaenia only in 4/41 1/41 demonstrated azotaemia but was observed to be azotaemic before therapy commenced anorexia in 3/41 dogs grand mal seizures and death in 1/41 haemorrhagic diarrhoea occurred in 2/41 dogs vomiting occurred in 27/41 dogs. This was observed within 6 hours of infusion. (thrombocytopaenia = $<100x10^{12}l^{-1}$) (granulocytopaenia = $<6x10^{9}l^{-1}$)

Table 19 (cont). Reported use of cisplatin in the dog and toxic side effects

Author	Date	Dose	Administration protocol	Monitoring	Toxic effects noted
LaRue and others	1989	1) i/a 70mg/m^2 (2 doses, 3 weeks apart) 2) i/a 70mg/m^2 with radiation therapy (as above with 25-40 Gy of radiation over 22 day period starting with first infusion) 3) i/v 10mg/m^2 with radiation therapy Monday, Wednesday, Friday over 22 day period with infusion directly before)	1) + 2) diuresis 24 hours before and 24 hours after infusion using lactated Ringers: 1st 8 hours 2.75ml/kg/hr 2nd 8 hours 4.12ml/kg/hr 3rd 8 hours 5.5ml/kg/hr during infusion 11-22ml/kg/hr post infusion 5.5ml/kg/hr cisplatin infused over 2 hours mannitol prior to infusion 50-100mg no anti-emetic (GA during infusion) 3) no infusion or drugs	assessment protocol not stated	1/8 dogs on i/v and radiotherapy arm developed increase in BUN and isothenuria after the second cisplatin cycle. This responded to conservative management. 1/8 dogs on the radiotherapy arm received 1 less cycle because of depression of WBC
Prydie	1991	70mg/m^2 at 21 days intervals. Number of cycles at least two probably four.	diuresis 4 hours before and 2 hours after with 0.9% saline at 18.3ml/kg/hr cisplatin given in 0.9% saline at the same rate dexamethasone intravenously (10mg) in combination with metoclopramide (20mg) subcutaneously given after problems experienced after 1st infusion.	assessment protocol not stated	vomiting persisting for two days after 1st infusion. Also anorexia and depression over that period. No reported problems with subsequent doses. no clinical evidence of bone marrow suppression or renal failure observed.

Table 19 (cont). Reported use of cisplatin in the dog and toxic side effects

Author	Date	Dose		Administration protocol	Monitoring	Toxic effects noted
Straw and others	1991	group 1)	70mg/m^2 18 days post-operatively and 21 days later	preceding 24 hours: -24 to -16 LRS @ 4.5ml/kg/hr -16to -8 LRS @ 6.75ml/kg/hr -8 to 0 LRS @ 9ml/kg/hr	not stated	mild vomiting four dogs (i in group 1, 3 in group 2) had to be hospitalised to control prolonged vomiting two animals had signs of myelosuppression with granulocytopaenia in one and granulocytopaenia and thrombocytopaenia in the other
		group 2)	70mg/m2 at time of histological diagnosis and 22 days later immediately post surgery	25% mannitol 50ml over 5-10 minutes cisplatin administered over 2 hours in a dextrose saline drip (500ml in total) + 2 to 26 hours LRS @ 27ml/kg/hr group 2) diuresis 4 hours before and 2 hours after with 0.9% saline at 18.3ml/kg/hr cisplatin given in 0.9% saline at the same rate		
Kraegel	1991	50mg m^2 cisplatin on 28 day cycle for about 6 cycles		diuresis of saline 12-18 hours before infusion at 105ml/m^2/hr 6 hours post infusion at the same rate mannitol administered before infusion (15mg/m^2 in 70ml saline/m^2/hr) metoclopramide given to some dogs	CBC, serum biochemistry, urinalysis, endogenous creatinine clearance sodium sulfinalate clearance (if urethral catheterisation not possible) were performed routinely before each monthly cycle	no clinical renal disease observed 1 dog developed exacerbation of an immune mediated thrombocytopaenia no reports of myelosuppression in other patients 1 dog required supportive therapy for gastrointestinal signs after 2 of the cycles administered

Table 19 (cont). Reported use of cisplatin in the dog and toxic side effects

ASSESSMENT OF PLATINUM

One of the aims of this project was to assess certain of the pharmacokinetic parameters of cisplatin in some of the patients managed with cisplatin chemotheapy at GUVS. This involved taking blood samples before, during and after drug infusion. These were analysed for their content of elemental platinum.

OBTAINING PLATINUM SAMPLES FROM PLASMA ULTRAFILTRATE:

The plasma samples were prepared from venous blood samples taken from a catheter placed in a peripheral vein before the beginning of the infusion. These were remote to the catheter utilised for the infusion of the chemotherapeutic and the administration of the other drugs used in the protocol.

On withdrawal the samples were stored in lithium heparin tubes (Starstent) to allow the preparation of plasma samples. These were held at room temperature until such a time as the plasma could be obtained and the samples centrifuged. This was normally within 2 hours and usually significantly shorter.

The platinum species were obtained using the Amicon Centrifree micropartition system[3]. This system is based around a micropore membrane that retains molecules > 25,000-30,000 Daltons (Amicon Ltd, personal communication) and effectively removes the proteins found in plasma (>99.9%, Amicon Ltd). Thus plasma bound drug is retained and the filtrate contains the unbound ligand. This technique was selected as it is technically straightforward and requires little laboratory time.

Though a variable volume of filtrate is produced it has been demonstrated that the concentration of solutes in this this is representative of their concentration in the protein free portion of the plasma sample (Amicon 1989). One millilitre aliquots of blood were taken from the lithium heparin tubes after agitation and centrifuged for 3 minutes at 15,000rpm in disposable polyethylene tubes. The system (see Figure 5) was charged with 500çl plasma and centrifuged at 3500 (approximately equivalent to 1000 xg) for 25 minutes at 5^{0}C. It was observed that a number of the samples were haemolysed. This was considered as a possible source of confusion in the analysis of the platinum levels. However, it is unlikely that haemolysis would contribute significantly to free platinum levels (except if bound to molecules of <25,000 Daltons) as little is present within the erythrocytes (Litterst and others 1976b) and that which is associated with these cells is likely to be irreversibly bound to components of the cell contents (McCanish and others 1981). Platinum bound to molecules of less than 25,000 Daltons would pass

[3]Amicon Ltd., UpperMill, Stonehouse, Gloucestershire, GL 102JB. (045-382-5181)

through the filter during centrifugation, however, these quantities are likely to have been small. This may be of relevance where only low levels of unbound platinum are present.

The protein free extract collected in the polyethylene cup, the top portion of the system was discarded and the cap applied. The specimens were stored at -20^{0}C until analysed.

A number of samples were mistakenly stored by the SURRC for a prolonged period at room temperature. This resulted in the loss of water, and as a result, though the platinum could be detected the actual concentration that was

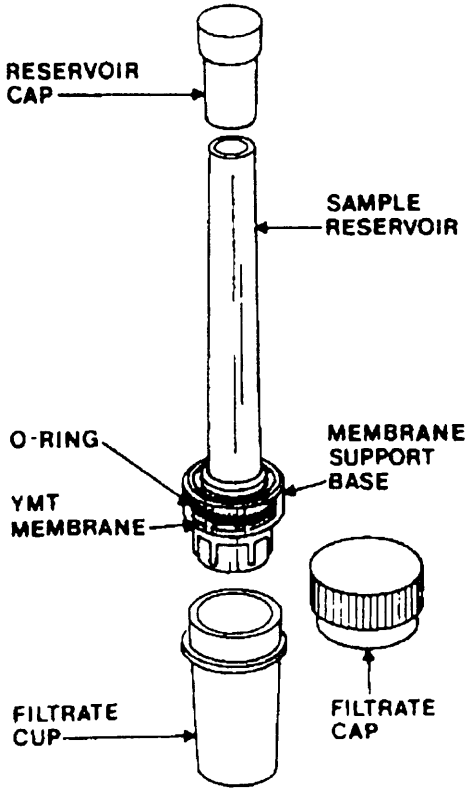

Figure 5. The component parts of the Amicon Centrifree system[4]

represented could not be calculated. As a result a considerable number of data points were lost, affecting final analysis of the infusions.

[4]Used with permission

METHODS AVAILABLE FOR PLATINUM ESTIMATION IN BIOLOGICAL SAMPLES

The method of estimating platinum concentration in tissues and body fluids has primarily been by variations of atomic absorption spectrophotometry. Litterst and others (1976) suggested a sensitivity of 10ng/ml (with estimates down to 3-4ng/ml). A recent assessment by Tothill and others (1990) for a graphite furnace atomic absorption spectrometer gave a the same limit of detection.

Currently the most sensitive system routinely used is the inductively coupled plasma mass spectrometer (ICP-MS). This is quoted to have a limit of detection of $0.1\mu gl^{-1}$ (equivalent to +/- 0.1ppb[5]) of platinum (Delves 1985, Tothill and others 1990). This is about a one hundred fold increase in sensitivity over atomic absorption spectrophotometery. The calculated accuracy for the samples submitted from the patients included in this thesis was +/- 0.2 ppb (or $0.2ngml^{-1}$)[6].

High performance liquid chromatography has been described having a sensitivity of 2.5ng/ml (Reece and others 1987). A recent suggestion for an equally accurate but cheaper method was made by Nygren and others (1990) using adsorptive voltammetry. This also has the advantage of avoiding the small degree of matrix effect that is found with the ICP-MS technology.

Problems occur in the determination of low levels of elements due to the matrix effect. This can be increased by the nitric acid used for tissue digests. This is greater for atomic absorption spectroscopy than it is for ICP-MS (Tothill and others 1990) and is the major factor for its lower resolution. Cross over may occur if samples with high concentrations are analysed proceeding ones with lower levels. Planning of the order of sample analysis, attention to cleaning and the use of internal standards are used to minimise these effects.

The major disadvantages with ICP-MS technology are the capital and running costs, which are substantial (the argon carrier gas is an expensive consumable).

OUTLINE OF THE TECHNOLOGY

The material to be analysed is presented in solution (this is made up in hydrochloric acid). The first procedure involves a nebuliser, the products of which are exposed to a high temperature inductively coupled plasma. This produces positively charged ions. These pass into a high vacuum quadrapole mass spectrometer which carries out multi-element analysis. Effectively the ions in the

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[5]ppb = parts per billion

[6]Ms T. Shimmied, SURRC, East Kilbride

plasma stream are separated according to the degree of deflection by the magnets. This is related to their atomic mass.

Specificity is high and interference from other elements is low especially at high atomic weights. This makes the technique ideal for the analysis of platinum (Tothill and others 1990). As the technique is very sensitive small samples can be managed.

The instrument used for these analyses was a VG Plamsaquad PQ1 fitted with a Fassel-type torch and an IBM PC-AT data system. It is housed in the Scottish Universities Research and Reactor centre, East Kilbride.

POSSIBLE SOURCES OF CONTAMINATION OF SAMPLES ANALYSED FOR PLATINUM CONTENT

In the analysis of samples for very low levels of a substance inadvertent contamination is an important factor in the consideration of the significance of the observations.

In this instance, the laboratories are unlikely to be a potential source of contamination as platinum is not utilised in either the surgery laboratory (preparation of plasma samples) or the anatomy, histology and animal husbandry laboratories (preparation of tissue samples). This a major worry in laboratories where platinum is regularly in use (Tothill[7], personal communication).

All containers are a potential source. Values are not quoted for the materials utilised in any of those used. It would be likely though that if they were a significant a constant low level would be detected.

The acids used are not considered a potential source of platinum (though they contribute to the matrix effect) (Tothill and others 1990). Though it is possible that the glassware is a source of platinum it is an unlikely in these laboratories and every effort was made to clean in an appropriate way. Washing with acid is adequate for preparation of samples for the assessment of low levels of other metals such as cadmium (McDonald, personal communication[8]).

Cross contamination between samples was reduced to a minimum by the use of disposable containers, pipette tips and centrifuge tubes. At the point of analysis quality control is rigid to reduce this problem.

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STABLISHMENT OF NATURALLY OCCURRING TISSUE PLATINUM CONCENTRATIONS

As far as the author is aware, there are no published figures for the levels of platinum in the tissues of dogs that have not been exposed to platinum. The use of ICP-MS technology on biological samples has only recently been described. Papers describing the distribution of platinum in canine tissue omit the assessment of the base line (Reviere and others 1990). This is probably related to the resolution available with the measuring techniques used. Naturally occurring platinum in human blood and tissues has been described in Australia using the equally sensitive technique of absorptive voltammetry (Nygren and others 1990). Up to the development of these techniques, platinum in organisms not exposed to platinum containing drugs could only be estimated using pooled sera, hair, nail clippings, etc. (Nygren and others 1990).

To assess the background levels of platinum plasma and tissue samples were obtained. Plasma from dogs not under going platinum chemotherapy was assessed using blood obtained at the time of sampling for clinical reasons. This blood was handled in the same way to that which was taken from animals during cisplatin chemotherapy (see page 60). Tissue samples were taken in the post mortem room of Glasgow University Veterinary School. Tissues were cut using the routine knives and saws available. Until they were further prepared they were stored in polyethylene containers at -20^{0}C.

Tissue samples were prepared for platinum analysis by acid digest to remove the organic components. The fresh weight of the samples was recorded and they were dried to a constant weight in an oven at 110^{0}C. Using glassware cleaned with nitric acid, samples were digested in 7 ml of Aristar nitric acid[9], heated at 98 ^{0}C, until dryness. Reconstitution was to 25 ml. Initially this was with de-ionised water. However, for later samples this was changed to 10% Aristar hydrochloric acid due to technical problems with the stability of the platinum if made up with water.

Individual exposure to cisplatin was unknown but the assumption that exposure had not occurred was made. As this drug is both expensive and currently very limited in its clinical veterinary usage this is not unreasonable (it would have been obvious if individual had platinum levels that suggested exposure to a source of administered therapeutic platinum).

The mean level of plasma ultrafiltratable platinum detected in controls was 0.35 (+/- 0.2) ng ml^{-1}. The detection accuracy of the technique is such that these levels are on the very limits of detection. They are such that it was deemed

[9]Aristar nitric acid analysis.

unnecessary to adjust the levels detected during the administration of the drug. At 24 hours, the limit to which the area under the curve was calculated, detected levels were in excess of 15x this level. The platinum levels from individual animals are presented in the appendices (Table 35, page 111).

Tissue samples (kidney, bone, liver, spleen and muscle) were taken from 5 carcasses at post mortem. No significant levels of platinum were detected.

SUMMARY:

The clinical use of cisplatin is not as extensive in the dog as it is in man. This is probably related to the expense, clinicians perceptions' of the practicalities of its administration and the potential for toxicity

In use, it has been shown that it can be administered safely with tolerable toxic effects. Though renal toxicity is perceived as the most important problem recent reports of its use suggest that, in canine oncology, myelosuppression is an important consideration. Dogs have not been recorded as suffering from ototoxicity and peripheral neurotoxicity both of which of are great clinical importance in man. This is probably because dogs are given lower doses and fewer cycles. This may change as the protocols evolve. Currently the recommendations appear to be changing to multiple doses.

Most of the effort in the design of administration regimes in man and the dog are directed towards renal protection. Little work has been done on myelotoxicity, though it appears that techniques that protect against renal damage do not protect against myelosuppression.

CHAPTER 4.

REVIEW OF THE LITERATURE PERTAINING TO THE MANAGEMENT OF OSTEOSARCOMA IN THE DOG

CLINICAL EXPERIENCE WITH CISPLATIN IN THE DOG

Cisplatin has to present had relatively limited clinical use in veterinary oncology in contrast to the human field where it is one of the most important drugs. Though it has been applied to a number of situations in the dog (Table 20, 68-72) it has found its greatest reported application to be in the management of osteosarcoma. Its efficacy has not been as dramatic in the veterinary field as it has been in human.

However, the use of cisplatin as a single chemotherapeutic agent, in conjunction with surgery, in the treatment of canine appendicular osteosarcoma has produced a consistent increase in patient survival. Currently median survival times are in the range 32-59 weeks. This compares with the survival period of 18-25 weeks for dogs managed by amputation alone. Though the use of historical controls in the analysis of the results of the management of human osteosarcoma has been questioned, the canine disease seems very consistent and their use is acceptable (Spodnick and others 1991).

PRESENT PROTOCOLS IN USE IN THE DOG FOR THE ADMINISTRATION OF CISPLATIN

Currently there is no agreed protocol for the clinical use of cisplatin in the dog. A number of reports of the use of cisplatin in a number of clinical situations has been described. These are detailed in Table 20 on pages 68-72. In general they all include the administration of fluids with preinfusion hydration and diuresis. The use of specific diuretics is less consistent. All papers state that the ideal protocol has yet to be defined.

Cisplatin is administered both intravenously (IV) and intra-arterially (IA) for regional perfusion of the tumour in conjunction with limb sparing. Though IA infusion increases the percentage tumour necrosis in comparison to IV administration there has been no demonstrated benefit in long term survival (LaRue and others 1989).

A number of authors report other chemotherapeutic regimes. These involve the use of drugs with accepted use in the treatment of osteosarcoma in humans. The use of doxorubicin as a single agent has been disappointing compared to cisplatin (Madewell and others 1978, Ogilvie and others 1989b). Treatment with methotrexate (with leucovorin rescue), cyclophosphamide and doxorubicin following amputation gave a median survival period of 60 weeks (range 12-180) (Henness and others 1977).

HYDRATION PROTOCOLS

Saline hydration and diuresis is identified as an important feature in the control of cisplatin induced renal toxicity and is a feature of all the reported clinical uses of cisplatin in the dog (Table 20, pages 68-72). Initially volumes of fluid administered were large and the time periods prolonged as experience was limited and clinicians were cautious. More streamlined protocols have been used more recently as confidence and experience have grown. A protocol described in experimental, healthy dogs by Oglivie and others (1988) using 0.9% saline administered at 18.3ml kg-1 for 4 hours and 2 hours after cisplatin infusion has become the basis of many of the current administration protocols.

LIMB SALVAGE SURGERY

THE IMMUNE RESPONSE TO CORTICAL ALLOGRAFTS IN THE DOG

The literature related to the use of massive cortical allografts in the management of fractures in the dog suggests that immune mediated response to allograft implantation does not represent a clinical problem with failures attributed mainly to infection and improper stabilisation of the graft (Henry and Wadsworth 1981b, Johnson 1988).

Recognition of bone graft rejection is not well described (Friedlaender 1991). Clinical and radiographic progress are not sensitive techniques for the assessment of immunological reactions in bone (Stevenson and others 1991) Incorporation of a graft is an active process of vascularisation, resorption and new bone formation. Radiographically this is seen as loss of trabecular detail and loss of density. This starts at the proximal and distal ends and spreads towards the centre of the graft. Graft-host union is usually seen 3-4 months after surgery. Rejection results in sequestration of the tissue or resorption without replacement (Johnson 1988).

There is evidence that like the transplantation of other tissues bone stimulates a response in the host (Friedlaender 1991). However, the low cellular content may result in the slow development of associated changes (Stevenson 1987).

Burchardt and others (1978) demonstrated that in general fresh allografts are apparently rejected and that freeze drying does not prevent this occurring but does reduce the magnitude. A similar observation was made by Stevenson (1987).

It has been suggested that the primary immunogenicity lies with the bone marrow cells that remain (Stevenson 1987). However, work in mice suggests that other components of bone may elicit a response (Horowitz and Friedlaender

1991). One study of the effect of freezing on immunogenicity involved the canine fibula, a bone having no marrow cavity in the adult dog (Burchardt and others 1978). Tissue matching of dogs significantly reduces the immune response induced by allogenic bone (Stevenson 1987). Twenty percent of fresh allografts in Burchardt and others series (1978) were incorporated in a way histologically indistinguishable from fresh autografts, presumably because of unintentional tissue matching.

The immune response appears to have two components :- a complement dependent cell mediated cytotoxicity and a humoral component. These are directed against class I and II histocompatibility antigens (Stevenson 1987). Freezing would appear to reduce the antibody dependent humoral response more than the antibody dependant cell mediated cytotoxic response (Stevenson 1987). Experimental immune suppression with azathioprine resulted in improved incorporation of canine fresh allografts again suggesting the immune response may be of significance (Burchardt and others 1977).

The activated T cell response to fresh murine bone allografts were cytotoxic $CD8^+$ and there appeared to be clones responding to both class I and class II antigens (Horowitz and Friedlaender 1991). This is an unusual response as $CD8^+$ T cells usually respond to class I antigens. These cells are known to be involved with the rejection of soft tissue grafts. This response occurred in the absence of bone marrow (a good source of both classes of histocompatibility antigens) though the cell types responsible for this stimulation are unknown. It is unlikely that the cell matrix or mineral are related to T cell activation as the antigens to which they respond are features of cell surfaces. The cells were possibly macrophages, which are a potent source of class II antigens (or osteoclasts, which might be expected to express class II antigens as they are of the same derivation).

The clinical significance of the immune reaction to allogenic bone in our patients is questionable where the major problems are apparently related to technical insufficiencies. It is possible though that the effects of an immune mediated response may underlie these apparently straight forward clinical problems. The author is unaware of tissue matching being used clinically for the assessment of patients undergoing allograft implantation.

Author	Date	Dose	Administration protocol	Situations utilised in	Outcome
Melhaff and others	1984	60mg/m^2 cisplatin on days 1 and 29 of a 42 day protocol given in combination with vindesine (0.1mg/kg)	saline diuresis for 12 hours pre-infusion (rate not stated). cisplatin infused over approximately 1 hour post-infusion saline diuresis maintained for 18 hours at pre-infusion level mannitol (2mg/kg) i/v for 20 minutes immediately before cisplatin	pulmonary carcinoma	2/2 showed >50% reduction of tumour volume one surviving 7 months and the other 19 months
Himsel and others	1986	initially 25mg/m^2 but raised to 60mg/m^2 given for one dose after apparent remission. No clinical effect until given at 40mg/m^2. second case 60mg/m^2 administered 3 weekly to effect	diuresis with 0.9% saline for 3 hours pre-infusion at 25ml/kg/hr. cisplatin administered over 20 minutes possibly separately post infusion fluids given at 12ml/kg/hr for 3 hours mannitol (18.75g/l saline) added to saline and continued through infusion and post infusion. chlorpromazine 0.5mg/kg i/v if vomiting occurred	metastatic squamous cell carcinoma	one PR for 6 weeks one CR died of chemotheraputic resistant lymphoma + 16 months

Table 20. Cisplatin chemotherapy protocols described in the literature.

Author	Date	Dose	Administration protocol	Situations utilised in	Outcome
Mauldin and others	1988	60mg/m^2 in combination with doxorubicin @30mg/m^2 given as two courses over 8 weeks (cumulative dose 60mg/m^2 doxorubicin and 120mg/m^2 cisplatin)	diuresis 30ml/kg 8 hours pre and 8 hours post cisplatin given over 8 hours mannitol @ 500mg/kg i/v dexamethasone @ 0.025mg/kg i/v prochlorpromerazine 0.1mg/kg i/v	osteosarcoma: appendicular disease amputation axial disease *enbloc* resection	appendicular osteosarcoma median survival time 43.8 weeks 1 year survival 7/19 (37%) disease free interval median 28.5 weeks cohort of amputation only medi survival 25 weeks 1 year survival 4/19 (21%) disease free interval median 22 weeks
Shapiro and others	1988a	50mg/m^2 over 6-8 hours at maintenance in saline (70ml/m2/hr) 40mg/m^2 if creatinine clearance <1 standard deviation below the mean 2-6 courses. evidence of metastatic disease no further treatment	diuresis of saline 12-18 hours before infusion at 105ml/m^2/hr (approximately 3.5ml/kg/hr) 6 hours post infusion at the same rate mannitol administered before infusion (15mg/m^2 in 70ml saline/m^2/hr) metoclopramide given to some dogs	appendicular osteosarcoma	median survival time 43 weeks (11) cohort survival 14.5 weeks (8)

Table 20 (cont). Cisplatin chemotherapy protocols described in the literature.

Author	Date	Dose	Administration protocol	Situations utilised in	Outcome
Shapiro and others	1988b	50mg/m^2 over 6-8 hours at maintaince in saline (70ml/m2/hr) 40mg/m^2 if creatinine clearance <1 standard deviation below the mean 2-6 courses. evidence of metastatic disease no further treatment	diuresis of saline 12-18 hours before infusion at 105ml/m^2/hr 6 hours post infusion at the same rate mannitol administered before infusion (15mg/m^2 in 70ml saline/m^2/hr) metoclopramide given to some dogs	transitional cell bladder tumour squamous cell carcinoma of the head and neck	transitional cell carcinoma 1/8 PR for 31 weeks 4/8 stable disease for 12,30,32, and 34 weeks squamous cell carcinoma 3/5 PR for 2,10, 15 weeks
Knapp and others	1988	60mg/m^2 variable number of courses depending on response: PR 3 weekly until relapse CR 3 weekly for 2 cycles after remission	diuresis 20ml/kg/hr 4 hours before and 2 hours after 0.9% saline 20 minute infusion in 0.9% saline no anti-emetic unless vomiting occurred no diuretic		an overall response rate (CR an PR) of 7/36 (19%) was observe CR was observed in 1/11 sq.cell carcinoma CR was observed in 1/1 with undifferentiated mediastinal carcinoma PR was observed 1/11 sq.cell carcinoma 2/3 metastatic osteosarcoma 1/3 nasal adenocarcinoma 1/1 thyroid carcinoma

Table 20 (cont). Cisplatin chemotherapy protocols described in the literature.

Author	Date	Dose	Administration protocol	Situations utilised in	Outcome
LaRue and others	1989	1) i/a 70mm/m^2 (2 doses, 3 weeks apart) 2) i/a 70mg/m^2 with radiation therapy (as above with 25-40 Gy of radiation over 22 day period starting with first infusion) 3) i/v 10mg/m^2 with radiation therapy Monday, Wednesday, Friday over 22 day period with infusion directly before) cisplatin administered before surgery	1) + 2) diuresis 24 hours before and 24 hours after infusion using lactated Ringer's: 1st 8 hours 2.75ml/kg/hr 2nd 8 hours 4.12ml/kg/hr 3rd 8 hours 5.5ml/kg/hr during infusion 11-22ml/kg/hr post infusion 5.5ml/kg/hr cisplatin infused over 2 hours mannitol prior to infusion 50-100mg no anti-emetic (GA during infusion) 3) no infusion or drugs	osteosarcoma of the appendicular skeleton suitable for reconstructive surgery	median survival time 32 weeks over all groups (range 4-180) 1 year survival time 7/20 (35%)
Prydie	1991	70mg/m^2 at 21 days intervals. Number of cycles at least two probably four. cisplatin administered before surgery	diuresis 4 hours before and 2 hours after with 0.9% saline at 18.3ml/kg/hr cisplatin given in 0.9% saline at the same rate dexamethasone intravenously (10mg) in combination with metoclopramide (20mg) subcutaneously given after problems experienced after 1st infusion.	osteosarcoma of the distal radius	alive at 12 weeks post diagnosis with no radiographic signs of pulmonary metastatic disease

Table 20 (cont). Cisplatin chemotherapy protocols described in the literature.

Author	Date	Dose		Administration protocol	Situations utilised in	Outcome
Straw and others	1991	group 1)	70mg/m^2 18 days postoperatively and 21 days later	preceding 24 hours: -24 to -16 LRS @ 4.5ml/kg/hr -16to -8 LRS @ 6.75ml/kg/hr -8 to 0 LRS @ 9ml/kg/hr	osteosarcoma of the distal limb	group 1) 37 weeks median survival , DFI of 32 and 38% 1 year survival
		group 2)	70mg/m2 at time of histologic diagnosis and 22 days later immediately post surgery	25% mannitol 50ml over 5-10 minutes cisplatin administered over 2 hours in a dextrose saline drip (500ml in total) + 2 to 26 hours LRS @ 27ml/kg/hr group 2) diuresis 4 hours before and 2 hours after with 0.9% saline at 18.3ml/kg/hr cisplatin given in 0.9% saline at the same rate		group 2) 40 weeks median survival, D of 25 and 43% 1 year survival there is no statistical difference between groups 1 and 2
Kraegal and others	1991	50mg/m2 every 4 weeks for 6 cycles or the detection of metastatic disease		diuresis of saline 12-18 hours before infusion at 105ml/m^2/hr (approximately 3.5ml/kg/hr) 6 hours post infusion at the same rate mannitol administered before infusion (15mg/m^2 in 70ml saline/m^2/hr) metoclopramide given to some dogs	appendicular osteosarcoma	median survival 59 weeks with 62% (10/16) surviving 1 year

Table 20 (cont). Cisplatin chemotherapy protocols described in the literature.

PR	partial response	50 % or greater decrease in volume with no increase in size of any other lesions or the development of other lesions
CR	complete response	remission
Survival time		Time from start of therapy to death (check individual reports because may vary)

LIMB SALVAGE EXPERIENCE REPORTED IN THE LITERATURE

Amputation, though the most effective way of controlling tumours locally, does not fulfil most surgeons' wish to engineer a full return to function. Techniques to reconstruct the defect and maintain limb function following en-bloc resection have been described in humans (Mankin 1987b). Two basic techniques are described:- the use of endoprostheses and the replacement of the resected bone with allografts. Procedures using osteochondral grafts have also been described (Mankin and others 1983). Different centres appear to use and advocate one or other of these methods.

Endoprostheses are not readily available for the canine but the potential for harvesting bone for the preparation of allografts is considerable. A number of centres in the USA have developed the use of allografts in the management of canine bone tumours. The majority of this work has been performed at the Colorado State University and the North Carolina State University.

A technique where the resected tumour was replaced with bone cement containing methotrexate was unsuccessful, with those animals not succumbing to metastatic disease requiring amputation of the limb because of complications with the technique (Hagege and Hernigou 1987).

A massive cortical graft can be used to reconstruct the defect left following tumour resection. There are numerous reports of the successful use of allografts in the management of fractures in animals (Henry and Wadsworth 1981a, Sinibaldi 1989). A cortical graft provides physical support, encourages osteoconduction and may stimulate osteoinduction. However, the concept of osteoinduction by grafts has been recently questioned and may not be a feature of the use of allografts in higher mammals (Schwartz and others 1991). There are no viable cells in these grafts to be transplanted. Incorporation of a cortical bone graft takes months to years and may never be completed. This means that during the period of incorporation it is significantly weaker than host bone. Removal of plates should not be contemplated within 1-2 years of grafting (Johnson 1988). A cancellous autograft is routinely utilised to encourage incorporation at the host interfaces.

A number of sites have been selected for limb sparing surgery. The most successful have been the distal radius, proximal humerus and distal tibia. Procedures at all these sites involves arthrodesis of the adjacent joint. Experience with management of tumours around the stifle is disappointing and at least one centre now longer attempts it (LaRue and others 1989).

The reported results suggest that if there are no complications, and post operative morbidity can be significant (25-35% in human patients within the first 3 years, Stevenson and others 1991), the expected outcome for this type of surgery is similar to that found with the arthrodesis of the same joint for other reasons. This

is suggested as a reason for the disappointing experiences with surgeries involving stifle arthrodesis. Straw and others (1990) report good to excellent limb function in 69% of their evaluated cases.

Complications include infection, failure of fixation and local regrowth. In the largest reported series of 70 dogs (Straw and others 1990) complications included a rate of local recurrence of 24% and an infection rate of 31%. Of the 22 animals with allograft infection 7 required amputation with the remainder responding to systemic or local antibiotics (gentamycin impregnated polymethyl methacrylate beads). These findings highlight the potential for a considerable degree of morbidity associated with limb sparing surgery, even at a centre which has developed considerable experience. Comparison of the survival of dogs under going limb sparing plus chemotherapy showed no difference from a group under going amputation and chemotherapy.

Mankin (1987b) reports an incidence of infection of 12.4% in a series of 209 human patients managed with allografts for a variety of conditions including 43 osteosarcomas. He notes that complications were found most commonly in patients under going vigorous chemotherapy post surgery. Currently reported cases in dogs involving allografts are of animals that have had only preoperative chemotherapy.

Graft incorporation was observed in animals without complications (LaRue and others 1989). Allograft infection was the primary cause of failure incorporation in a series of 12 animals in which the progress was monitored

Neither of these American centres utilised post operative support (other than a Robert-Jones dressing for a couple of days to control swelling). How this aspect of management of these animals compares with their routine management of arthrodesis patients is not stated. Recommendations for the post operative management of a carpal arthrodesis include the use of a cast for 6 weeks post surgery (Newton and Nunamaker 1984). The use of a cast following a distal joint arthrodesis is routine at GUVS.

The staging of the tumour (Enneking system, page 8) at the time of presentation is significant, with 1A and 1B tumours having a success rate of 70% with this dropping to IIA and IIB tumours having a success rate of 30-40%. Most tumours in dogs are staged as IIB (Straw and others 1990).

Degree of tumour necrosis and prognosis

The use of preoperative chemotherapy is common in the management of human osteosarcomas. The primary reason is to reduce the bulk of the tumour to aid the surgeon at the time of resection. The degree of necrosis of the tumour at the time of surgery has been assessed as a prognostic indicator. Lane and Glasser

(1987) describe techniques in which the resected tumour is examined for necrosis - prognosis improves with increasing degree of necrosis. It is also used to assess the chemotherapeutic regime and adjust it if necessary.

This relationship between tumour necrosis and survival has not been demonstrated in the dog (Powers and others 1991). However, it is strongly predictive of the likelihood of local recurrence with 80% necrosis being the watershed. Less than 80% necrosis is associated with a high liklihood of local regrowth. There are differences between the chemotherapy strategies in dog and man, which may be related to this apparent difference in the predictive value of tumour necrosis following preoperative chemotherapy (particularly the use of post-operative chemotherapy). Animals given radiotherapy of the primary alone would not expect to have their time to metastasis extended if micrometastasis was already present, which might be expected.

Tumour necrosis was related to the method of preoperative management. Canine osteosarcomas are usually spindle cell tumours and as such might be expected to be relatively chemo- and radio-resistant thus relatively high doses of radiation were administered. Radiotherapy gave the greatest tumour kill (81.6%) with a combination of radiotherapy followed by intra-arterial cisplatin (83.7%). Radiotherapy potentiated the response of the tumour to cisplatin. There was no difference in necrosis between untreated tumours and tumours managed with intravenous cisplatin (approximately 23%).

Radiotherapy alone is not to be recommended. Not only does it not control established metastatic disease but it also interferes with allograft incorporation resulting in increased failure rates (Thrall and others 1990).

Effect of cisplatin administration on graft incorporation

Cisplatin has a potent effect on DNA that is not cell cycle dependent, being expressed as cell death at mitosis (Shapiro 1989). A recent study in rats has shown it to inhibit the incorporation of allografts with vascularisation retarded and new bone production reduced compared to untreated controls. Depression of the bone marrow (a well recognised cisplatin induced toxicity) and its osteoprogenitor constituents may be partially responsible for this effect (Zart and others 1991[1]).

The contribution of chemotherapy to the post-operative morbidity of patients receiving allografts has been alluded to. However, in the management of the metastatic disease it is routine to administer repeated cycles to humans. Thus far it has not been so in the dog (Straw and others 1990). The reason for limiting

[1]Dr. S. Stevenson, Department of Orthopaedics, Case Western Reserve University, 2074 Abington Road, Cleveland, Ohio 44106, USA.

the number of cycles in canine osteosarcoma therapy is not discussed (a number of protocols for other tumours have described repeated cycles as well as some of the osteosarcoma protocols).

SUMMARY

Cisplatin has not attained the reputation and use in the dog that it has in human oncology. This may be related to the considerable cost of the drug, caution because of its reputation for toxicity, rejection because of the effort required in administration and the less dramatic clinical effects achieved. However, it undoubtedly increases the survival of patients with a number of sarcomas including osteosarcoma. The full value of cisplatin chemotherapy in the dog has probably still to be established.

It has been demonstrated that it can be administered with safety and minimal toxicity. The toxicity associated with long term administration in humans have not been described in canine patient, probably because of the relatively short courses and lower doses. However, even the short term diuresis regime described by Ogilvie and others (1988) requires a substantial investment in time and materials.

The use of cisplatin in osteosarcoma has extended the lives of patients from the 18-25 weeks that might be expected following palliative amputation. Currently median survival periods of 32-59 weeks are reported (Table 20, pages 68-72). This increase in survival has been observed with periods of chemotherapy that are short compared to man. It is possible the survival period may be increased by increasing the number of cycles administed. Occasional individuals appear to have very long periods of remission reminiscent of the animals that are apparently cured by amputation.

CHAPTER 5.

ANALYSIS OF RESULTS

This chapter summarises the clinical outcome and pharmacokinetics of the platinum in plasma of a number of cases of appendicular osteosarcoma treated in the Surgery Department at Glasgow University Veterinary School. Not all cases treated had samples taken for platinum analysis.

HANDLING OF CISPLATIN

In common with many chemotherapeutics cisplatin is a hazard to personnel who handle it. It is known to be a mutagen, carcinogen, teratogen and an irritant to mucus membranes (Swanson 1988). Though these effects have not been quantified in humans (Lederle Laboratories 1986) (though observed in animal experiments) it is considered unacceptable to wait for the accumulation of such evidence in the human population before suggesting precautions to be taken whilst working with it (Blue Sheet Drug Report 22, 1979 cited by Swanson 1988).

Cisplatin is made available as a lyophilised powder in glass vials (10mg and 50mg) with rubber seals[1]. It is reconstituted by the introduction of water for injection. Pressure rises in the bottle during this process and there is a danger of aerolisation as the needle is withdrawn. Care was taken to equalise the pressure before withdrawal of the syringe by aspirating gas into the syringe used to introduce the water. Whilst handling the drug staff wore a surgical mask and latex gloves.

It is known that platinum containing metabolites of the drug are excreted in the urine. In many centres drug infusion is carried out in a metabolic cage to make management of the excreta more straight forward. Such facilities were not available at GUVS and in an effort to control soiling kennels were generously bedded with newspaper. Patients were given frequent opportunities to void urine in the run. Contaminated newspaper was handled by staff wearing heavy duty rubber gloves. Urine passed in the run was washed away with copious amounts of water.

ADMINISTRATION OF CISPLATIN

Steel needles were used to add water for rehydration of the drug (aluminium inactivates cisplatin, Lederle Laboratories 1986). This was done immediately before infusion as cisplatin has limited stability once reconstituted (Lederle Laboratories 1986). The calculated dose of cisplatin was made up in sufficient 0.9% saline to give a 20 minute infusion at the rate of 10ml/kg/hr.

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[1]Cisplatin, Lederale laboratries, Cyanamid of Great Britian Ltd., Fareham Road, Gosport, Hampshire, PO13 0AS.

Patients were not fed on the morning of infusion but were allowed free access to drinking water. To minimise the chance of catheters being pulled free during the infusion excitable patients were sedated, using a cocktail of acepromazine[2] (0.05mg/kg) and buprenorphine[3] (0.01mg/kg) given intramuscularly. This was repeated if required. Patients from whom samples for platinum analysis were collected were not sedated.

Prior to infusion patients were hydrated with 0.9% saline for 4 hours at a rate of 10ml/kg/hr. Fluids were administered using a Travenol[4] Flo-Guard 6200 peristaltic infusion pump. At the end of this period they were exercised for a brief period to allow urination.

Prior to infusion one of two diuretics was administered:

- mannitol, 0.5g/kg as a slow intravenous injection
- frusemide (Lasix, Hoechst[5]) 0.5mg/kg intravenously

followed by the anti-emetic metoclopramide (Emequell, Beechams[6]) 1mg/kg by slow intravenous injection.

Following infusion of the cisplatin fluid administration was continued for a further 2 hours at 10ml/kg/hr. Patients were fed approximately 6 hours post infusion.

MEASUREMENT OF RENAL FUNCTION

It was felt that an estimation of renal function would be useful as it was intended to give multiple doses of the drug. Clinical experience in dogs has shown decreasing renal function with repeated doses (Shapiro and others 1988a). Calvert and others (1989) had demonstrated the dramatic effect that renal insufficiency could have on the pharmacokinetics of carboplatin.

Creatinine clearance has been shown to be an insensitive technique in this type of work in humans and has been discredited. The current recommendations are for a technique involving ^{51}Cr-EDTA which has been demonstrated to be very sensitive (Daugaard and Abildgaard 1989).

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[2]ACP, C-Vet Ltd., Bury St. Edmunds, Norfolk

[3]Temgesic, Reckitt and Colman, Dansom Lane, Hull HU8 7DS

[4]Travenol Laboratories Ltd., Thetford, Norfolk.

[5]Lasix, Hoechst UK Ltd., Pharmaceutical Division, Hoechst House, Salsibury Road, Honslow, Middlesex, TW4 6JH.

[6]Emequell, Beecham Research Laboratories, Great Western Road, Brentford, Middlesex, TW8 9BD.

Initially plans were made to measure creatinine clearance as this was the only functional test feasible in the clinical situation at GUVS. This was to be a "short" version performed over a period of 3 hours as a metabolic cage required for the 24 hour technique was not available. A number of trial runs were made to assess the practicality of this technique and it was shown to be so wildly variable that it was abandoned.

There is a also a problem with creatinine clearance in male dogs in which the secretion of creatinine into the tubules can lead to overestimation of GFR (Chew and Dibartola 1989). This can be accommodated for in the calculations but compromises further a technique that is insensitive. As renal mass decreases secretion increases making the estimation of GFR less accurate as renal disease advances.

As the labelled chromium technique was not available in the Department it was elected not to pursue renal function. Blood urea and serum creatinine were measured as a guide to severe renal dysfunction (a rise in these parameters implies 75% of the nephrons are compromised).

SELECTION OF PATIENTS

The selection of patients to be included within a clinical trial can dramatically influence the outcome (McEwan 1989b). Individuals were selected with respect to the following criteria:

- histologically confirmed osteosarcoma (biopsy)
- no other pathology that might be expected to reduce remaining life span to less than one year
- no sign of disseminated neoplastic disease (survey radiographs of the thorax and abdomen)
- renal function not significantly impaired (page 79)
- no apparent impairment bone marrow function (routine haematology)
- client assessment

Table 21. Selection criteria for patients managed with cisplatin at Glasgow University Veterinary School.

AIMS OF INVESTIGATION

The ideal dose regime of cisplatin in both man and the dog has yet to be established. Though clinical efficacy can be compared between regimes(see conclusions), a thorough understanding of the pharmacokinetics of the drug in the treatment of canine osteosarcoma has yet to be considered.

Currently drug dose appears to be the largest physical dose that results in acceptable toxicity. There is a steep dose-response curve with a number of tumour types but increasing dose is complicated by toxicity. There is evidence that prolonged exposure may be more relevant than peak levels achieved (Drewinko and others 1973, Gandara and others 1989). The area under the concentration-time curve for prolonged infusions as opposed to push infusions is greater with decreased urinary excretion suggesting greater body retention (Gullo and others 1980, Saito and others 1990).

The area under the concentration-time curve (AUC) is used in pharmacology as a measure of exposure to a drug. As cisplatin is effectively irreversibly protein bound it was decided to assess the AUC for the unbound fraction of the drug. It was felt that the AUC might be a useful parameter in the comparison of administration protocols. It is known that for a given protocol the AUC shares a linear relationship with the dose administered (Riviere and others 1990). Peak levels of platinum were also assessed as these have been related to nephrotoxicity (Campbell and others 1983). Platinum was measured between cycles, where possible, for evidence of persistence.

Platinum concentration is used as an estimate of cisplatin concentration. The two parameters are not directly related as cisplatin undergoes aquation into a number of derivatives in the plasma. Using techniques that specifically identify cisplatin it is thought that during the 2 hours immediately post infusion the platinum in the ultrafiltrate represents unchanged drug but by 4 hours the bulk of the platinum detected is in the form of aquation products (Reece and others 1987). In the rat it has been shown that the proportion of cisplatin that is present in the pool of platinum in the plasma ultrafiltrate after 3 hours is in the order of 1% of that at 15 minutes after injection (Daley-Yates 1985).

The fate of cisplatin administered to these patients cannot be followed as there is no information on total plasma platinum or excretion. If these parameters are followed in conjunction a picture of tissue take-up can be established (Dumas and others 1990).

Blood sampling intervals

The elimination of plasma platinum from the body of dogs following the administration of cisplatin has been shown to be bi-phasic with a $t_{1/2}$ α of less than 1 hour and $t_{1/2}$ β of 4-5 days. Within 4 hours following an intravenous bolus plasma levels were 90% reduced and 60-70% of the administered dose was recovered in the urine. There was little change in the plasma platinum concentration between day 4 and day 12 (Litterst and others 1976). The decay of ultrafiltratable platinum in humans following infusion of cisplatin has been described Table 18 (page 43). It varies with the dose and the protocol of administration but is the region of 20-30 minutes.

The intention was to assesses the AUC for the platinum in the plasma ultrafiltrate achieved by our administration protocol. The author was not aware of information pertaining to what to expect for the administration of this dose as an infusion in the dog. Experimental work with cisplatin tends to involve bolus administration. However, it appeared likely that the greatest changes would be observed during the infusion and in the four hours following it. Little change over the period 24 hours to 3 weeks was expected. It was not certain that platinum would be detected in the plasma ultrafiltrate during this period as the plasma platinum might be expected to be protein bound. Samples during this period were taken in conjunction with routine samples required for haematological monitoring. The time intervals selected are presented in Table 23 (page 84).

DATA FROM CISPLATIN ADMINISTRATION TO PATIENTS AT GLASGOW UNIVERSITY VETERINARY SCHOOL

PATIENT DETAILS

Patient details for cases of primary bone tumours managed with cisplatin are summarised in Table 22 (page 83). This series of dogs have similar characteristics to those reported in the literature. With one exception (case number (110232) they were large and giant breed dogs, with an mean weight of 40 kg. They were mostly male (5/7). The mean age was 6.9 years. There were 6 osteosarcomas and 1 chondrosarcoma. The tumours were classified as IIB (with one exception, case number 110809 which was classified as IB) using the Enneking system.

Case Number	Breed	Age (years)	Sex	Weight (kg)	Tumour type	WHO staging	Enneking staging	Histological staging	Disease free interval (weeks)	Survival (weeks)
110232	cross-bred	11	male	21.5	osteosarcoma distal radius	T3M0	IIB	high grade spindle cell	14.6	unknown
110809	Saint Bernard	9	male	60	osteosarcoma distal radius	T3M0	IB	low grade spindle cell	25.9	34.3
112509	Great Dane	6	female	48	osteosarcoma distal radius	T3M0	IIB	high grade spindle cell	7.4	11.3
114324	labrador	6	male neuter	36	osteosarcoma distal radius	T3M0	IIB	high grade spindle cell	19.1	22.7
114993	old English sheepdog	5	male neuter	42	osteosarcoma distal tibia	T3M0	IIB	high grade spindle cell	52.6	59.9
115930	German shepherd dog	5	male	36	osteosarcoma proximal humerus	T3M0	IIB	high grade spindle cell	12.9	21.1
116799	German shepherd dog	6	female	36.5	chondrosarcoma proximal humerus	T3M0	IIB	high grade spindle cell	7.7	19.4
	mean	**6.9**		**40.0**				**median**	**16.8***	**22.7***

Table 22. Signalment of cases of appendicular osteosarcoma treated at Glasgow University Veterinary School.

* for cases: 110809, 112509, 114324, 114993, 115930, 116799.

	Time (minutes)	Time (hours and weeks)
start of infusion	0	
	2	
	5	
	10	
	15	
end of infusion	20	
decay period	2	
	5	
	10	
	15	
	30	
	60	1 hour
	120	2
	240	4
	360	6
	480	8
	720	12
	1440	24
	10080	1 week
	20160	2
	30240	3

Table 23. Time points selected for the assessment of the decay of ultrafiltratable platinum after administration of cisplatin as a 20 minute infusion.

PHARMACOKINETICS OF PLATINUM IN THE PLASMA ULTRAFILTRATE

The plasma ultrafiltrate platinum concentrations for these patients are presented in the appendices (Tables 36-38, pages 112-118) and as semi-log graphs (Figures 6-10, pages 88-92). The peak concentrations are in Table 24 and the pharmacokinetic data in Table 26 (page 87).

Case Number	Cycle	Dose of cisplatin (mg m^{-2})	Peak [free platinum] ng ml^{-1}
114993	1	60	2916.50
	2	40	882.40
	3	40	1249.80
	4	40	information not available
115930	1	60	1340.69
	2	40	information not available
116799	1	40	1597.25
	2	60	2078.36

Table 24. Peak concentrations of free platinum in the plasma of patients given cisplatin chemotherapy.

PHARMACOKINETIC DATA

Peak concentrations were observed around the end of the cisplatin infusion. The mean peak concentration at 40 mg m^{-2} was 1451.95 ng ml^{-1} plasma (range 882.40-1597.25) and at 60 mg m^{-2} was 2128.59 ng ml^{-1} (range 1340.69-2916.50).

The pharmacokinetic analysis of the ultrafiltratable platinum concentrations was performed by Dr A. Kellman using a software package Status3[7]. Visual inspection of the decay curves suggests a three exponential decay. However, analysis of the data was unable to accommodate the long terminal half life into a decay model. The graph in Figure 7 has been plotted to include the long terminal halflife. This was the only instance where the computer model would accept it. The best fit was to a two exponential model with a mean $t_{1/2}\,\alpha$ for 40 mg m^{-2} of 9.5 minutes (range 1.9-15.1) $t_{1/2}\beta$ for 40 mg m^{-2} of 29.2 minutes (range 18.1-40.4).

[7]Status3, Dr.A.Kellman, Department of Medecine and Theraputics, Glasgow University Medical School

The AUC for the ultrafiltratable plasma platinum concentration curve is consistent for 40mg m^{-2} at 4.7 x 10^4 ng min^{-1} ml^{-1} (range 4.3-4.9). The AUC, is as expected, increased by an increase in the dose (Riviere and others 1990). A 50% increase in the dose of cisplatin resulted in 80% increase in the AUC.

The k10 represents the rate at which the platinum is eliminated from the central compartment, and assumes that all drug elimination occurs via this compartment. It remains reasonably constant across the individual cycles.

The clearance represents the volume of plasma cleared of platinum per unit time. For individuals this would appear to be remarkably consistent and over the cycles in all the patients it appears to remain fairly constant.

The volume at a steady state (Vdss) is achieved when the rate of administration equals the rate of elimination and the compartments are in equilibrium. The mean value of Vdss for the ultrafiltratable platinum after 40 mg m^{-2} 430 ml kg^{-1} (range 430-890).

The apparent volume of distribution is related to distribution through the body. Drugs that are unable to leave the central compartment, for example as the result of binding, have a smaller volume than those that can penetrate other tissues as will those that are concentrated for any reason. The volume will also be increased by tissue take up. Very lipid soluble drugs, for example many injectable anaesthetic agents, have a very high volume distribution suggesting concentration.

PERSISTENCE OF PLATINUM

Analysis of samples taken at periods of 1, 2 and 3 weeks post infusion showed the persistence of platinum in the plasma ultrafiltrate (Table 25).

Time (weeks)	Average, with standard deviation, and range of platinum concentrations in plasma ultrafiltrate (ng ml^{-1})
1	3.43 +/- 1.48(1.52-4.75)
2	1.86 +/- 1.06(0.65-3.05)
3	1.74 +/- 1.46(0.55-4.29)

Table 25. Persistence of platinum in the plasma ultrafiltrate.

Case number 116799 was sampled 6 weeks post chemotherapy at which a level of 1.49 ng ml^{-1} was detected. This suggests that the decay slows further after 1 week. It must be remembered that some of these samples were haemolysed (see page 58).

Case number	Cycle	Dose (mg m^{-2})	Dose administered (mg)	Weight (kg)	$t_{1/2}\alpha$ (minutes)	$t_{1/2}\hat{O}$ (minutes)	AUC (ng min l^{-1})	k10 (min^{-1})	clearance (l min^{-1} kg^{-1})	Vdss (l kg^{-1})
116799	1	40	36	34	10.4	32.4	$4.95x10^4$	0.044	0.014	0.43
	2	60	60	33	1.9	18.4	$8.582x10^4$	0.071	0.014	0.33
114993	1	60	70	38.5	information not available					
	2	40	45	37.8	15.1	40.4	$4.318x10^4$	0.024	0.018	0.89
	3	40	45	38.5	9.6	18.1	$4.651x10^4$	0.047	0.016	0.37
	4	40	45	41.3	information not available					
115903	1	60	65	36	information not available					
	2	40	34	36	3.0	25.7	$4.884x10^4$	0.085	0.014	0.39
average*					9.5	29.2	$4.70x10^4$	0.054	0.015	0.52
standard deviation*					4.9	9.5	$0.29x10^4$	0.024	0.002	0.25

Table 26. Some aspects of the pharmacokinetics of platinum in the plasma ultrafiltrate in the cases studied-Area under the concentration-time curve, exponential half lives of the decay, k10, clearance and Vdss.

* for doses of 40mg m^2

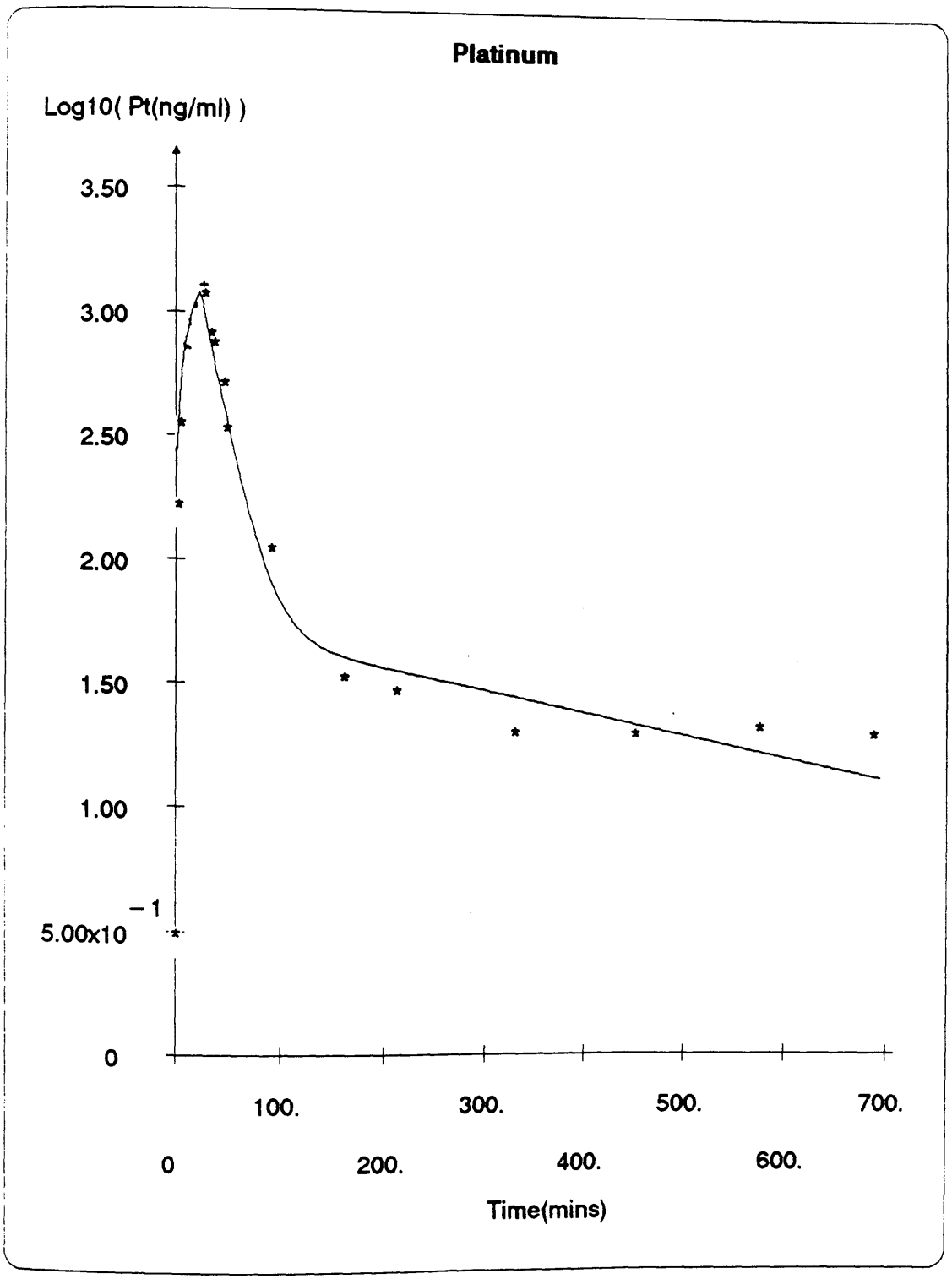


Figure 6. Log graph of plasma ultrafiltrate platinum concentration for case number 114993. Cisplatin @ 40mg m^{-2}, 2^{nd} cycle.

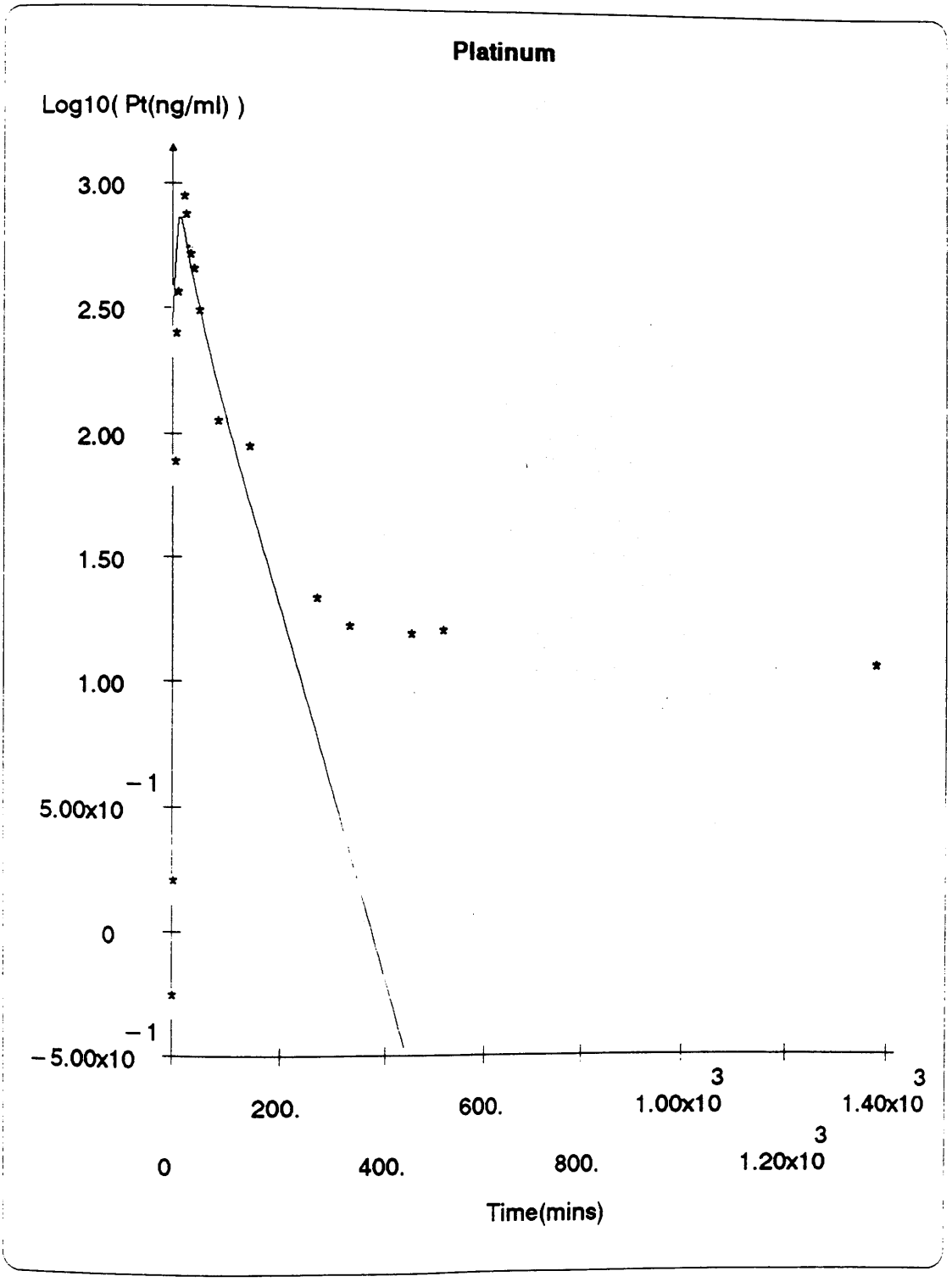


Figure 7. Log graph of plasma ultrafiltrate platinum concentration for case number 114993. Cisplatin @ 40mg m^{-2}, 3rd cycle

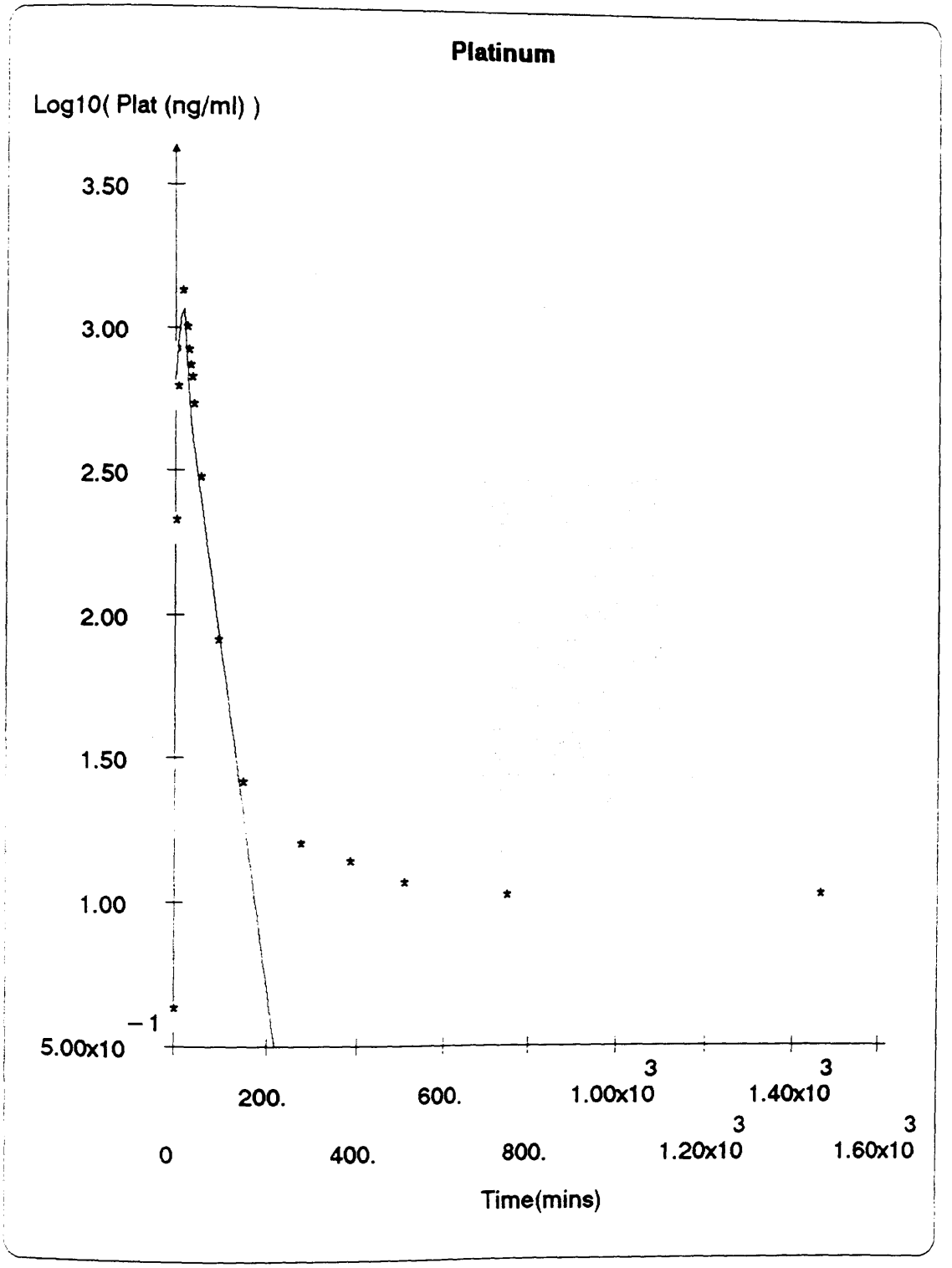


Figure 8. Log graph of plasma ultrafiltrate platinum concentration for case number 115930. Cisplatin @ 60 mg m^{-2}, 1st cycle.

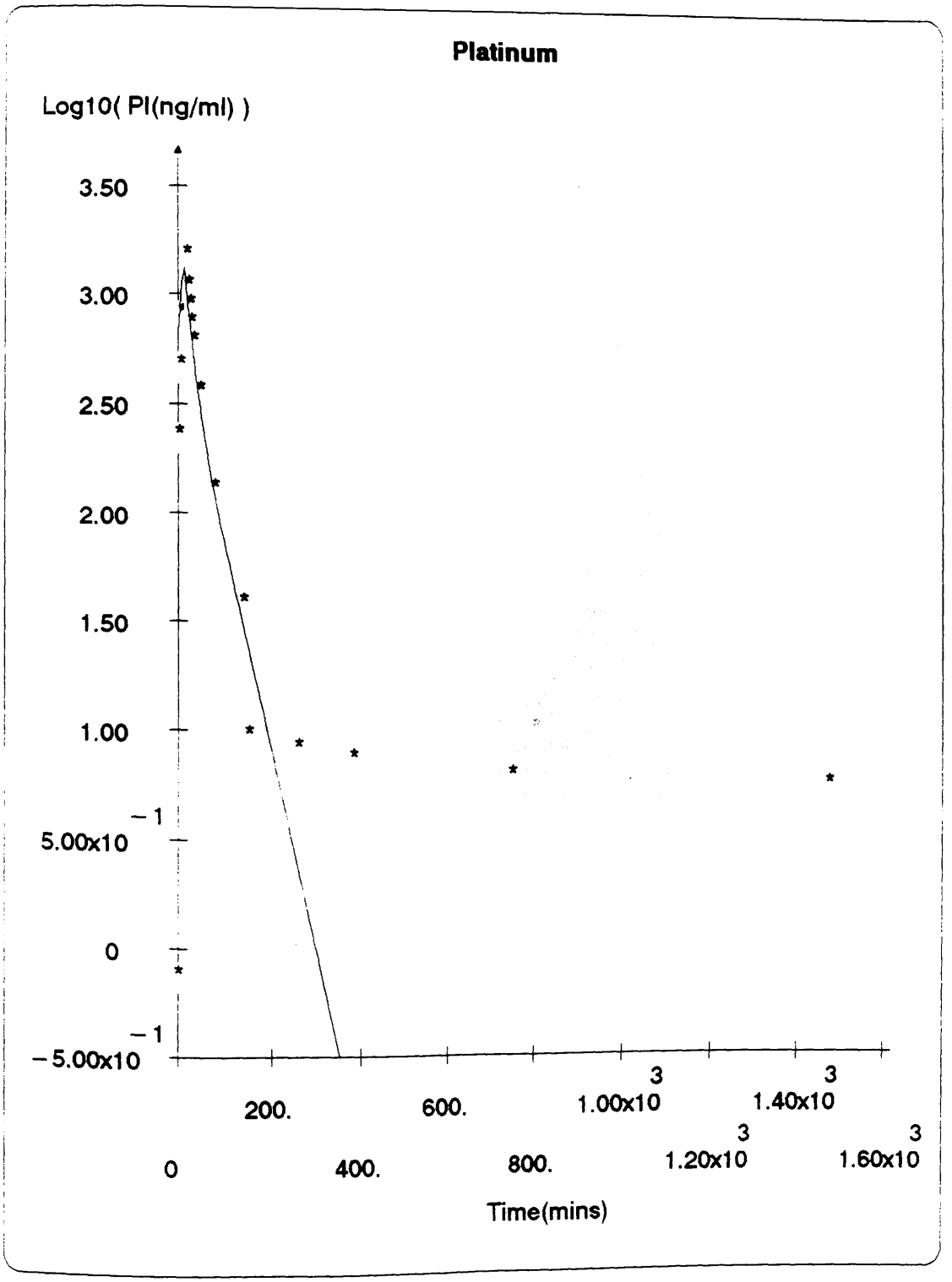


Figure 9. Log graph of plasma ultrafiltrate platinum concentration for case number 116799. Cisplatin @ 40mg m^{-2}, 1st cycle.

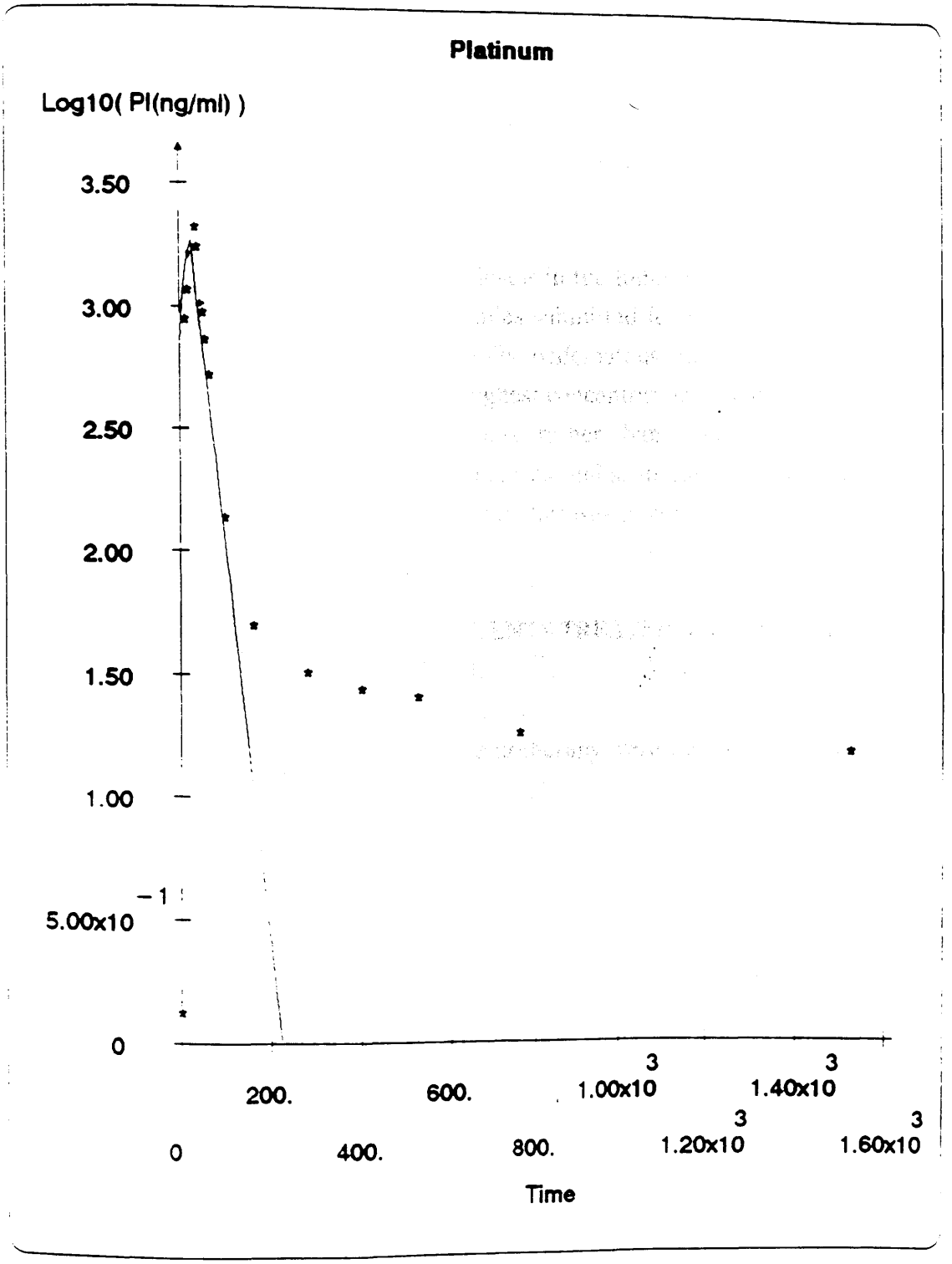


Figure 10. Log graph of plasma ultrafiltrate platinum concentration for case number 116799. Cisplatin @ 60 mg m^{-2}, 2^{nd} cycle.

TISSUE LEVELS

Platinum tissue levels are recorded from case number 115930 (Tables 27 and 28, page 95). This dog was managed with a preoperative dose of cisplatin followed by amputation and chemotherapy 32 days later. It was euthanased 143 days after diagnosis, which was 105 days after the second course of cisplatin, due to pain resulting from metastasis to the proximal femur. Tissue samples were analysed for platinum concentrations from the leg at the time of amputation and from the carcass at post mortem.

Interestingly the tissue platinum levels in the tumour at resection vary by a factor of 2. Sections taken of the samples submitted for analysis show the area with the lowest platinum concentration to be oedematous and have a dense plasma cell infiltrate of the tumour, whilst the highest concentration was an area with little infiltrate and probably periosteal response rather than tumour[8]. Distribution amongst the normal tissues at post mortem was similar to that described by Litterst and others (1976). There is a trend for metastases to contain less platinum than the surrounding parenchyma.

TOXIC EFFECTS OBSERVED IN PATIENTS TREATED AT GLASGOW UNIVERSITY VETERINARY SCHOOL

Patients undergoing cisplatin chemotherapy were monitored for toxic side effects of the drug. This involved:

1. weekly assessment of routine haematology
2. weekly assessment of routine biochemistry
3. observation for potential gastrointestinal problems
4. urinalysis if renal problem suspected

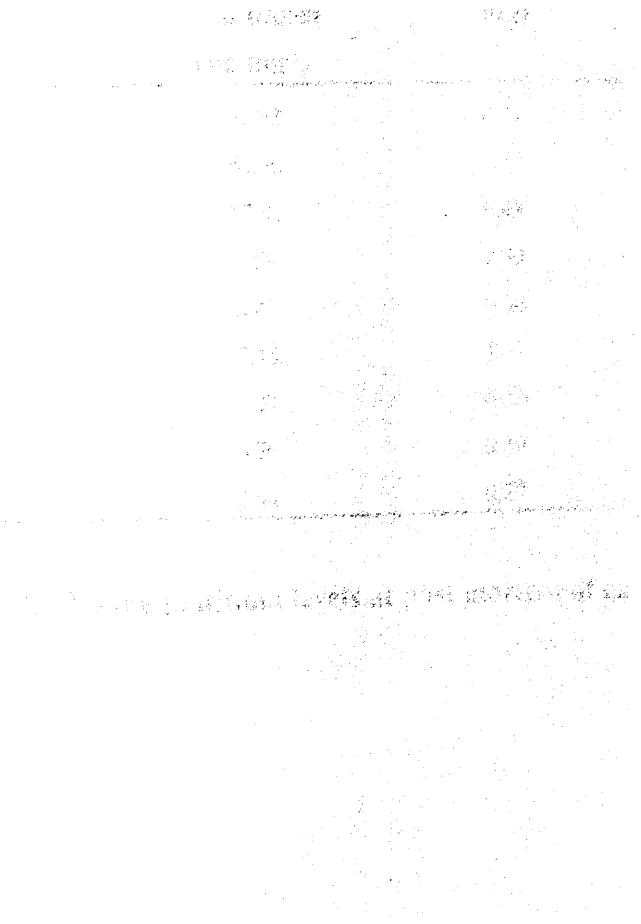
This routine was modified for individuals not within a convenient distance of GUVS. These animals were not sampled until presented for chemotherapy at which time haematology and biochemistry were reassessed before chemotherapy administration. Renal function *per se* was not assessed.

The toxic effects observed in these patients is summarised in Table 29 on page 96 to 98. Toxicity was encountered in the management of all cases (5) for which suitable data is recorded (Table 29). This was mild in one individual (110809, limited to vomiting) but was significant enough to lead to reduction of the

[8]Professor H. Pirie, Department of Veterinary Pathology, Glasgow University Veterinary School

dose in the other four cases. Renal toxicity was only a clinical evident in one case (115903) and recovered in time. Myelosuppression was the most significant side effect. Both granulocytopaenia and thrombocytopaenia were noted (114324, 114993, 115903, 116799). These did not necessarily occur concurrently or in every cycle given to an individual. Granulocytopaenia was defined as a total neutrophil count of less than $3x10^{9}l^{-1}$ and thrombocytopaenia as less than $200x10^{12}$-1. Granulocytopaenia was complicated with infection in one instance (114993). Management with intravenous antibiotics (amoxycillin with clavulanic acid[9]) and supportive fluids was effective.

No other significant side effects were noted.



[9]Augmentin, Beecham Research Laboratories, Great Western Road, Brentford, Middlesex, TW8 9BD.

Sample	Pt concentration in acid digest ($ng\ mg^{-1}$)	Dry weight (mg)	Pt concentration in sample dry weight ($ng\ mg^{-1}$)
tumour (1)	8.53	0.99	215.40
tumour (2)	13.04	1.92	169.88
tumour (3)	6.21	0.44	355.26
normal muscle	8.74	1.97	110.86
normal bone	10.44	1.69	154.80

Table 27. Platinum concentrations in tissue samples from the foreleg of case number 115930 taken at the time of amputation.

Sample	Pt concentration in acid digest ($ng\ mg^{-1}$)	Dry weight (mg)	Pt concentration in sample dry weight ($ng\ mg^{-1}$)
kidney normal	51.83	0.31	4179.84
liver normal	41.86	0.37	2867.12
spleen normal	17.81	0.43	1033.06
lung normal	6.59	0.30	543.73
muscle normal	6.42	0.54	298.33
bone normal	7.45	1.67	111.79
liver met	1.27	0.03	1094.83
spleen met	0.76	0.39	49.35
lung met	0.44	0.27	40.29

Table 28. Tissue platinum levels at post mortem of case number 115930.

Case number	Cisplatin doses	Renal toxicity	Myelosuppression	Gasterointestinal toxicity
116799	2 cycles: 1) 40mg/m^2 2) 60mg/m^2	no renal toxicity observed	1. granulocytopaenia which resolved after 3 weeks. transient suppression of platelet count. 2. granulocytopaenia persisting 3 weeks after infusion. Platelet count suppression more profound than after 1st infusion.	no vomiting but reported to have reduced appetite after 2nd infusion.
114324	3 cycles: 1) 60mg/m^2 2) 60mg/m^2 3) 60mg/m^2	4) transient rise in BUN and creatinine 1 week after infusion. No associated clinical problem. Resolved 10 days later.	1) panleukopaenia persisting at 3 weeks post infusion resulting in postponement of 2nd cycle. 2) granulocytopaenia persisting 18 days after infusion. Improved after further 2 days to allow 2nd cycle. 3) mild lympopaenia was recorded 1 week after infusion. This resolved. no suppression of the platelet count was documented.	vomiting after infusion if fed within a couple of hours of the end of fluids.

Table 29. Toxicity in cases of osteosarcoma managed with cisplatin at Glasgow University Veterinary School

Case number	Cisplatin doses	Renal toxicity	Myelosuppression	Gasterointestinal toxicity
114993	4 cycles: 1 60mg/m^2 2 40mg/m^2 3 40mg/m^2 4 40mg/m^2	no renal toxicity observed	1) granulocytopaenia and thrombocytopaenia. This resolved after 18 days 2) granulocytopaenia detected on day 15 that persisted to next infusion 21 days later. There was no thrombocytopaenia detected during this period. 3) granulocytopaenia persisted for at least 22 days but had resolved by 28 days. There was no thrombocytopaenia detected during this period. 4) granulocytopaenia persisted for at least 35 days. Occasional instances of granulocytopaenia were observed over the following month. There was no detected thrombocytopaenia.	vomiting was observed if food was not withheld for about 5 hours after the end of the infusion.

Table 29 (cont). Toxicity in cases of osteosarcoma managed with cisplatin at Glasgow University Veterinary School

Case number	Cisplatin doses	Renal toxicity	Myelosuppression	Gasterointestinal toxicity
115930	2 cycles: 1) 60mg/m^2 2) 40mg/m^2	1) acute renal failure	1) no abnormalities observed 2) granulocytopaenia detected on day 7 and persisted for at least 14 days. There was no thrombocytopaenia observed.	no vomiting was observed.
110809	8 cycles: 1) 60mg/m^2 2) 60mg/m^2 3) 60mg/m^2 4) 60mg/m^2 5) 60mg/m^2 6) 60mg/m^2 7) 60mg/m^2 8) 60mg/m^2	no evidence of nephropathy observed	mildly anaemic before chemotherapy and throughout management	vomiting was noted occasionally after infusion. This was reduced if food was with held for approximately 4-6 hours after infusion

Table 29 (cont). Toxicity in cases of osteosarcoma managed with cisplatin at Glasgow University Veterinary School

SURVIVAL BENEFITS OF CISPLATIN CHEMOTHERAPEUTIC PROTOCOL

It is important to realise that the disease free interval is a function of the investigative process (time to presentation, frequency of check radiographs, etc.) and that the survival time is influenced by the clients attitude to the timing of euthanasia.

Interval	Definition
disease free interval	time from date of biopsy confirming lesion to detectable metastasis
survival	time from date of biopsy confirming lesion to death

Table 30. Definition of disease free interval (DFI) and survival used in this dissertation.

The details of patients managed for primary bone tumours at GUVS, using a combination of surgery and cisplatin chemotherapy, are presented in Table 22 (page 83). All tumours were reviewed "blind" by a veterinary pathologist and classified on cellular morphology after Rosens's criteria (Table 6, page 9)[10]. They were osteosarcomas, with the exception of case number 116799 which was classified as a chondrosarcoma. All the osteosarcomas, with one exception, were classified as high grade spindle cell sarcomas. The chondrosarcoma was classified as a high grade spindle cell sarcoma.

Disease free interval (median, weeks)	Survival time (median, weeks)
16.8	22.7

Table 31. Survival and disease free interval for cases of osteosarcoma managed surgery and chemotherapy at Glasgow University Veterinary School.

The median disease free interval was 16.8 weeks (range 7.4-52.6) and the median survival period was 22.7 (range 11.3-75.3) (Table 31). Quoted survival periods in the literature range from 32-59 weeks.

Case number 110809 had sequential material available for histological examination :- the original biopsy at the time of diagnosis; a second biopsy from a suspected regrowth; material taken at post mortem. The initial material was less

[10]Dr. I.A.P. McCandlish, Department of Veterianry Pathology, Glasgow University Veterinary School

aggressive compared to samples examined from other cases and was classified as a low grade spindle cell sarcoma. Later material had a progressively more aggressive appearance.

EXPERIENCES WITH LIMB SALVAGE AT GLASGOW UNIVERSITY VETERINARY SCHOOL

Five patients underwent limb salvage surgery. Animals with suitable tumours of either the distal radius or tibia were selected. The criteria for the selection of patients were similar to those described by Straw and others 1990 (Table 32).

- confirmed osteosarcoma of distal radius or tibia with minimal soft tissue involvement
- not involving greater than 50% of diaphyseal length
- no evidence of metastatic disease
- no evidence of complicating or life threatening systemic disease
- owners who were committed to the procedure and aware of potential problems

Table 32. Criteria for the selection of patients for limb sparing surgery.

A bone bank was established following the recommendations of Johnson (1988). Allografts were obtained from retired racing greyhounds. These were to be culled because of chronic injuries and had been donated to the Department of Surgery. Donors were assessed by clinical examination and routine haematology and biochemistry samples were evaluated. Animals showing signs of significant local or systemic disease were rejected. It was elected to use the femur as it was felt that it would be the most useful considering the range of size in potential patients.

Donors were anaesthetised and prepared for aseptic surgery. At the time of draping the animal was euthanased with an overdose of barbiturate[11] given intravenously. The femurs were approached routinely and the bone exposed. It was divided at the two metaphyses and the diaphysis removed. This was denuded of soft tissues, periosteum and bone marrow with a rasp. A routine bacteriology swab was submitted for culture. Grafts were double wrapped in sterile bags, radiographed for the bone bank library and deep frozen. Storage was at -20^{0}C for a maximum of 6 months.

[11]Euthatal, RMB Animal Health, Rainham Road South, Dagenham, Essex RM10 7XS.

At the time of surgery grafts were defrosted at room temperature. Tumours were resected form the distal joint to a point 5 cm proximal to the extent of the changes identified on the radiograph (LaRue and others 1989). This was measured on the patients bone from the joint surface. Grafts were cut to size. They were stabilised in the defect with a dynamic compression plate used in a loaded fashion[12]. Care was taken to engage the recommended number of cortices proximal and distal to maximise stability (Sinibaldi 1989).

Patients were supported in a Robert-Jones dressing for the immediate post-operative period until the swelling had subsided. They were managed subsequently in a resin impregnated cast[13] for periods of around 10 weeks. The decision on whether to remove the cast was made on radiographic progress of graft incorporation. Three of the patients remained in an external support because of the development of complications.

Graft incorporation occurred in 4 cases. This tended to happen at the distal end first. This was identified radiographically as confluence of the trabecular pattern. Grafts were united in the period 6-19 weeks. The body of the grafts showed, at a varying rates, loss of detail in trabecular pattern and cortex definition. These changes are summarised in Table 33 (page 102).

COMPLICATIONS

Four of the five patients managed with allograft incorporation developed problems associated with their grafts. These problems included infection, local recurrence and fixation failure.

Infection

Three of the five allografts showed evidence of infection case numbers 110809, 114324, 114993). This was delayed in onset occurring between 8 and 16 weeks after surgery.

The classic signs of infection with local swelling, pain and discharge from the area were seen. Radiographic changes were obvious in the host bone. The graft appeared to respond by an increased rate of lysis that was focal in nature. As two of these cases also experienced local regrowth interpretation is difficult (case numbers 110809 and 114993). The development of infection led to the late diagnosis of the regrowth

[12]Synthes, 20 Tewin Road, Welwyn Garden City, Hertfordshire, AL7 1LG

[13]Scotchcast 2, 3M,

Local recurrence

Evidence of local recurrence was observed in two cases. In both cases this was of clinical significance. In one case (110809) this was managed by fore quarter amputation as there was no evidence of metastasis at the time of diagnosis. In the other case (114993) the regrowth was discovered at post mortem (the limb having under gone multiple surgeries for chronic infection).

Fixation failure

Fixation failure, other than related to allograft infection and regrowth, was seen in a Great Dane (case number 112509). This occurred rapidly, within 4 weeks of surgery, at which time the radius and ulna were markedly porotic and deformed under loading by the implants. This animal died of possibly unrelated reasons before the significance of this on her long term survival could be assessed (myelosuppression is a possibility as death was reported to be as a result of a lung infection approximately 10 days post chemotherapy, in contact dogs also succumbed). However, it would be fair to assume it was likely to have been significant.

Proximal implant loosening also occurred in two other cases. Infection and local regrowth were present in both instances.

Pattern of metastasis

Three dogs underwent post mortem examination the distribution of metastatic disease found is summarised in Table 34. Two animals exhibited metastasis to organs other than the lungs (case numbers 114903 and 115930). One animal, case number 115930, had a metastatic deposit in the proximal femur.

Case number	Distribution of metastatic deposits
110809	local recurrence at amputation site (at the time of amputation there was tumour present in the axillary lymph node) extending into mediastinum, multiple deposits in lungs, no macroscopic deposits identified elsewhere
114903	local recurrence at site of tumour resection, lungs, liver, spleen, both kidneys, adrenal
115930	lungs, liver, spleen, kidneys, proximal femur

Table 34. Distribution of metastatic disease in cases which underwent post mortem.

Case number	Assesment of graft incorporation		
	proximal	"body"	distal
110232	signs of bridging at 9 wweks with incorporation by 19 weeks	progressive loss of cortical bone visable from 1 week post implantation	incorporation at 12 weeks
110809	cuff of host bone developing by 10 weeks but development of local regrowth disrupted the progression of graft incorporation	loss of cortical bone first	incorporation caudally by 12 weeks
112509	no host reaction observed by 4 weeks at which time the proximal stabilisation failed	no change observed	no incorporation observed
114324	host cuff developed by 16 weeks with some incorporation at 19 weeks	slow loss of cortical bone not observed until 6 weeks post implantation	patchy incorporation by 12 weeks with incorporation by 16 weeks
114993	early incorporation at 9 weeks this was destroyed by active infection	mild cortical loss observed at 2 weeks that progressed slowly until infection became established	incorporation at 6 weeks

Table 33. Radiographic progression of allografts.

SUMMARY

The median survival periods of reported series of osteosarcoma range from 32-59 weeks (Table 20, pages 68-72). The survival periods achieved with this protocol are not as encouraging (median 22.7 weeks, range 11.8-75.3). These figures are based on a small number of patients and it is possible that with the treatment of greater numbers the survival period may be longer. Case numbers 110809 and 114993 had an encouraging post operative survival periods. The significance of tumour recurrence after limb salvage is uncertain with respect to the development of metastatic disease is uncertain. However, metastasis to the axillary lymph node in case number 110809 was probably significant in its survival period.

The area under the concentration-time curve (AUC) was remarkably consistent at a dose rate of 40 mg m^2 and was increased with a higher dose. Peak platinum concentrations were much more variable. As this value seems to change rapidly more frequent sampling might be important around the end of the infusion to establish the maximum value. Interestingly, the patient who was apparently exposed to the greatest peak in free platinum suffered the greatest degree of myelosuppression. The AUCs calculated for these cases are considerably higher than those for human patients given substantially greater doses ($4x10^4$ as opposed to 0.08 ng $min\ l^{-1}$). The reason for this is not known. It is possible that it is related to the method of calculation.

The effect of dose on parameters such as clearance and elimination half lives was not investigated. However, examination of Table 26 (page 87) suggests that these are fairly constant for the range of doses used here. The half lives observed in these patients are similar to that seen in man (Table 18, page 43). Clearance (mean 0.015l $min^{-1}\ kg^{-1}$) is similar to that reported by Hardie and others (1991) for dogs who recorded the equivalent of 0.017l $min^{-1}\ kg^{-1}$ after an intravenous dose of 90 mg m^{-2} given over 5 minutes. The Vdss (mean 0.52l $min^{-1}\ kg^{-1}$) is also in a similar range to that reported in that experimental study where it was quoted as the equivalent of 0.614l kg^{-1}. The Vdss of the platinum in the plasma ultrafiltrate is greater than that of strongly bound drugs such as flunixin (0.14l kg^{-1}, McKellar and others 1990) and less than that of a lipid soluble drug such as propofol (>3l kg^{-1}, Nolan and Reid 1991).

The persistence of platinum in the plasma ultrafiltrate has not been described before in the dog. The history of the platinum measured is uncertain. It is thought that the platinum binds to constituents of the plasma smaller than protein (which pass through the filtration cones). Whether the detected platinum relates to drug complexes formed at the time of infusion, bound to a substance

with a long half life, or whether these levels are related to the turn over of substances to which the platinum bound is unknown.

Platinum distribution in the carcass of case number 115930 was similar to that reported in experimental animals. Metastatic deposits in this case exhibit a trend for containing less platinum than the surrounding parenchyma. This suggests that they have been exposed to less platinum and thus presumably developed after the last cisplatin cycle. In contrast, one deposit (in the liver) has high platinum levels suggesting its presence at the time of at least one cycle.

The administration protocol used in these cases resulted in significant myelosuppression at 40 and 60 mg m^2. Clinically evident renal toxicity occurred in one patient and a transient rise in BUN and creatinine were observed after doses of 60 mg m^2. There are a number of reports of larger doses in the literature involving both greater and lesser rates of fluid administration which have not been associated with this degree of myelotoxicity. It is in the understanding of findings such as these that the publication of pharmacokinetic data with reports of clinical usage would be useful.

Few of the cases were necropsied. However, two of the three animals that were exhibited the wider pattern of metastasis that is associated with chemotherapy in both man and animals.

Limb salvage surgery at GUVS was associated with a high rate of morbidity. Reports of limb salvage surgery in both man and the dog suggest that if it is successful patients do well, but complications such as infection and regrowth lead to multiple procedures and in many cases amputation. Reported rates of regrowth are much higher in the dog than man. Morbidity associated with this type of surgery even in experienced centres can be high. The goals of management of this disease are to eliminate the tumour and the metastatic deposits that are an early development in its natural history. If limb salvage techniques are associated with a high incidence of local recurrence the first goal is not being achieved (Winkler 1991).

CONCLUSIONS

The aims of the work presented in this dissertation were:

1. to assess the benefit of cisplatin chemotherapy, as a method of the management of metastatic disease in the treatment of canine osteosarcoma, in the clinic of Glasgow University Veterinary School
2. a preliminary investigation of the pharmacokinetics of cisplatin administration in the clinical situation (the area under the platinum in plasma ultrafiltrate concentration-time curve) and its potential as a method of comparing therapeutic protocols between centres
3. to assess the technique of long bone reconstruction, following tumour resection, with allografts in conjunction with cisplatin chemotherapy

With any study of this nature, due to the limited number of clinical cases, it is difficult to make broad generalisations or recommendations. The following conclusions are however pertinent.

Comparing the outcome of cancer chemotherapy trials conducted at different centres

The comparison of clinical trials conducted at different centres is fraught with complications. Ultimately the most important feature is the survival period. The best statistical parameters to compare are survival curves (McEwan 1989b) (e.g. the Kaplan-Meier curve), rather than averages, though these are not routinely quoted at present in the veterinary literature. The median is a more useful average than the mean and is currently the most quoted statistic and was thus used in this study.

Ideally, the cohorts of animals compared should be matched for factors such as age, tumour staging, etc. For instance, it has been shown that old and young animals have a generally poorer prognosis than middle aged individuals (Spodnick and others 1991). There is evidence that the metastatic potential varies between individual tumours in the dog, though this has not been well characterised and it is possible that there may be a variation in sensitivity to cisplatin. As the understanding of the behaviour of osteosarcoma becomes better understood the more complex will become the analysis of its management.

The interpretation of results across multiple centres would benefit from a routine trial design. Currently this is not the case for osteosarcoma though some large collaborative groups exist in North America.

Factors that may be of significance in the design of a chemotherapeutic regime for cisplatin in the dog

Dose size and intensity have been shown to be important considerations in the design of therapeutic protocols in man. The ideals have yet to be established in the dog. Examination of Table 20 (pages 68-72) suggests that frequency of administration may be more significant than dose with the greatest median survival periods following multiple cycles of cisplatin. A dose of 50 mg m^{-2} given as 6 cycles appears to be more efficacious than 70mg m^{-2} given as 2 cycles. The very long infusions used in man (5 days) are probably not a practical proposition in the dog.

The vehicle of administration is significant. The use of saline stabilises cisplatin chemically reducing aquation reactions in the plasma. This reduces the production of potentially toxic products. The use of hypertonic saline is significant in not only allowing greater doses to be used (renal protection, though still potential problems with the other toxic effects in humans) but also encourages tissue uptake which is probably relevant therapeutically.

Time of administration would appear to be important both with respect to renal toxicity and increasing retention of platinum. The most advantageous time is the afternoon. The practicalities of the current standard protocol (Ogilvie and others 1988) with a 4 hour pre-hydration period means that this is the most convenient time as well. This is likely to encourage standardisation.

Renal toxicity is now well controlled through the design of administration protocol. Myelosuppression would appear to be a greater clinical problem. At present there are no techniques for ameliorating this problem in the dog other than reducing the dose administered.

The distribution of cisplatin following intravenous administration is not ideal in that the take-up by the lungs is not as great as other tissues. As the lung is the primary area for metastatic disease techniques to maximise this may be useful, though the relationship between tissue platinum levels and metastatic cytotoxicity is unknown. Whole body hyperthermia alters the distribution of platinum in favour of the lungs (Riviere and others 1990). The distribution to tissues can also be manipulated by altering the character of the cisplatin infusion. An increasing exponential infusion will give significantly higher concentrations of platinum in the lungs compared to a bolus injection (Page, personal communication[1]).

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The use of the area under the concentration-time curve (AUC) for platinum in plasma ultrafiltrate in the analysis of cisplatin administration protocols

In isolation this information is of limited value. It will give an impression of whether tissue exposure has been of a similar order but gives no information on the retention of platinum by the tissues.

Ideally the AUC for platinum in the plasma ultrafiltrate would be correlated with the AUC for the total plasma platinum and the amount of platinum excreted in the urine to give a fuller picture of its fate. In the clinical situation this would involve significant manipulations of the patient.

A simpler clinical assessment of exposure may be the concept of dose intensity described by Gandara and others (1989). This can be easily calculated from reports of clinical trials.

The use of cisplatin in the therapy of osteosarcoma and associated metastatic disease in man and the dog

Cisplatin is a chemotherapeutic of major clinical importance in man. However, though it is used in the therapy of osteosarcoma it is used as a component of a multiple drug regime. The major clinical uses of cisplatin are in the treatment of germinal cell tumours and solid carcinomas of the head and neck.

Currently cisplatin is the most commonly reported chemotherapeutic agent used for the therapy of canine osteosarcoma. It is reported almost exclusively as a single agent. There is no doubt that the use of cisplatin significantly increases the survival of dogs affected by the tumour. However, ultimately all patients die or are euthanased as the result of metastatic disease (there are occasional reports of animals with an apparent cure).

Improvements in the benefits of this kind of therapy for osteosarcoma lie with:

- assessing the tumour and its metastases for evidence of subsets with distinct biological behaviour
- improving the therapeutic index of cisplatin by the use of techniques such as hypertonic saline and other protective measures particularly with reference to myelosuppression
- introducing other drugs into the protocol
- assessing protocols by developing multi-centre prospective trials to increase case accrual rates
- achieving a better understanding of the pharmacokinetics in the clinical setting and relating this to outcome

- further investigation of the consequences of cisplatin chemotherapy on allografts used in the reconstruction of tumour resection sites

Though cisplatin does not, at present, prevent the inevitable consequences of canine osteosarcoma it does have positive benefits and it is likely that these can be improved by refinement of the administration protocol and dosing regime. For committed owners of affected animals, that are suitable for therapy, it is an option that should be considered and the ramifications carefully discussed.

APPENDICES

Patient identification	Platinum (ng ml^{-1})
117100	0.39
117113	0.64
117748	0.29
117740	0.58
117728	0.25
117677	0.47
113358	0.13
117482	0.34
117837	0.40
117844	0.00
mean	**0.35**
standard deviation	0.19

Table 35. Ultrafiltrable plasma platinum levels detected in 10 dogs hospitalised at Glasgow University Veterianry School and not exposed to cisplatin

Start and end of infusion	Sample number	Date and time	Time (minutes)	Pt (ng ml^{-1})
end	1	11/10/90 15:25	0	2916.50
	2	11/10/90 15:31	6	2216.50
	3	11/10/90 15:36	11	1797.00
	4	11/10/90 15:44	19	1288.50
	5	11/10/90 15:56	31	833.00
	6	11/10/90 16:39	74	161.50
	7	11/10/90 17:28	123	60.50
	8	11/10/90 19:28	243	32.50
	9	11/10/90 21:26	361	32.00
	10	11/10/90 23:23	478	31.00
	11	11/10/90 3:27	722	24.50
	12	11/10/90 15:25	1440	22.00

Table 36. Platinum in plasmsa ultrafiltrate for case number 114993.

Start and end of infusion	Sample number	Date and time	Time (minutes)	Pt (ng ml^{-1})
start	1	22/11/90 16:02	0	0.55
	4	22/11/90 16:04	2	1.60
	5	22/11/90 16:06	4	76.64
	6	22/11/90 16:09	7	248.94
	7	22/11/90 16:12	10	362.23
	9	22/11/90 16:17	15	796.02
end	10	22/11/90 16:21	19	838.40
	11	22/11/90 16:24	22	882.37
	12	22/11/90 16:27	25	744.77
	13	22/11/90 16:31	29	538.75
	14	22/11/90 16:35	33	516.40
	15	22/11/90 16:41	39	449.89
	16	22/11/90 16:51	49	305.48
	17	22/11/90 17:27	85	111.90
	18	22/11/90 18:24	142	88.71
	19	22/11/90 20:30	268	21.75
	20	22/11/90 21:30	328	16.80
	21	22/11/90 23:37	448	15.68
	22	23/11/90 01.34	512	16.08
	23	23/11/90 15:00	1378	11.91
	24	29/11/90 9:30	9688	4.47
	25	6/12/90 9:00	19738	1.47

Table 36 (cont.). Platinum in plasma ultrafiltrate for case number 114993.

Start and end of infusion	Sample number	Date and time	Time (mins)	Pt (ng ml^{-1})
start	k0	13/12/90 14:53	0	3.10
	k1	13/12/90 14:55	2	165.57
	k2	13/12/90 14:58	5	349.96
	k3	13/12/90 15:04	11	723.82
	k4	13/12/90 15:09	16	904.35
	k5	13/12/90 15:16	21	1062.78
end	k6	13/12/90 15:21	26	1249.80
	k7	13/12/90 15:23	28	1176.67
	k8	13/12/90 15:29	34	810.40
	k9	13/12/90 15:31	37	743.41
	k10	13/12/90 15:40	46	511.33
	k11	13/12/90 15:43	49	333.89
	k12	13/12/90 16:23	91	110.72
	k13	13/12/90 17:34	161	33.28
	k14	13/12/90 18:32	212	29.37
	k15	13/12/90 20:29	329	20.00
	k16	13/12/90 22:25	452	19.87
	k17	14/12/90 00:28	576	21.18
	k18	14/12/90 2:20	688	19.87
	k19	14/12/90 15:38	1505	2.89
	k20	19/12/90 8:56		destroyed
	k26			467.02
	k27			destroyed
	k28			459.87
	k29			282.70

Table 36 (cont.). Platinum in plasma ultrafiltrate for case number 114993.

Start and end of infusion	Sample number	Date and time	Time (minutes)	Pt (ng ml^{-1})
start	k2-1	10/1/91 14:03		destroyed
	k2-2	10/1/91 14:05	2	200.14
	k2-3	10/1/91 14:10	7	534.16
	k2-4	10/1/91 14:16	13	962.95
end	k2-5	10/1/91 14:25	22	953.74
	k2-6	10/1/91 14:28		destroyed
	k2-7	10/1/91 14:31		destroyed
	k2-8	10/1/91 14:36		destroyed
	k2-9	10/1/91 14:41		destroyed
	k2-10	10/1/91 14:58		destroyed
	k2-11	10/1/91 15:52		37.53
	k2-12	10/1/91 16:27		39.20
	k2-13	10/1/91 18:33		6.40
	k2-14	10/1/91 20:33		destroyed
	k2-15	10/1/91 22:27		12.50
	k2-16	11/1/91 2:23	740	12.28
	k2-17	11/1/91 14:24	1461	15.98
	rk18/1/91	18/1/91 9:29		4.75
	rk25/1/91	25/1/91 9:30		3.05
	rk31/1/91	31/1/91 15:50		1.41
	rk 7/2/91	7/2/91 11:10		1.27

Table 36 (cont.). Platinum in plasma ultrafiltrate for case number 114993.

Start and end of infusion	Sample number	Date and time	Time (minutes)	Pt (ng ml^{-1})
start	s1	20/12/90 14:37	0	0.65
	s2	20/12/90 14:39	2	442.24
	s3	20/12/90 14:42	5	1007.26
	s4	20/12/90 14:47	10	1734.51
	s5	20/12/90 14:53	16	2502.96
end	s6	20/12/90 14:56	19	1962.00
	s7	20/12/90 15:02	25	1657.53
	s8	20/12/90 15:06	29	1756.00
	s9	20/12/90 15:11	36	430.00
	s10	20/12/90 15:23	48	324.00
	s11	20/12/90 15:36	51	417.00
	s12	20/12/90 16:06	81	241.00
	s13	20/12/90 16:56	131	50.49
	s14	20/12/90 20:00	255	61.63
	s15	20/12/90 21:12	327	27.58
	s16	20/12/90 22:57	432	33.69
	s17	21/12/90 3:59	803	12.99
	s18	21/12/90 15:14	1477	8.04
	s19	27/12/90 10:25	9828	2.16
	ks5/1/91	3/1/91 12:05	20008	1.75
start	s2-1	11/1/91 16:58	0	4.29
	s2-2	11/1/91 17:00	2	214.41
	s2-3	11/1/91 17:04	6	618.28
	s2-4	11/1/91 17:09	11	840.76
	s2-5	11/1/91 17:13	15	1340.69
end	s2-6	11/1/91 17:23	25	1002.88
	s2-7	11/1/91 17:26	28	834.07
	s2-8	11/1/91 17:29	31	destroyed
	s2-9	11/1/91 17:33	35	destroyed
	s2-10	11/1/91 17:38	40	destroyed
	s2-11	11/1/91 17:53	55	destroyed
	s2-12	11/1/91 18:30	92	destroyed
	s2-13	11/1/91 19:25	147	destroyed
	s2-14	11/1/91 21:31	273	destroyed
	s2-15	11/1/91 23:21	383	destroyed
	s2-16	12/1/91 1:24	506	destroyed
	s2-17	12/1/91 5:27	749	destroyed
	s2-18	12/1/91 17:23	1465	destroyed
	18/1/91	18/1/91 9:47	9660	4.22
	1/2/91	1/2/91 16:16	30198	1.03

Table 37. Platinum in plasma ultrafiltrate for case number 115930

Start and end of infusion	Sample number	Date and time	Time (minutes)	Pt (ng ml^{-1})
start	v1	25/04/91 14-30	0	0.80
	v2	25/04/91 14-32	2	239.29
	v3	25/04/91 14-35	5	497.64
	v4	25/04/91 14-40	10	860.46
	v5	25/04/91 14-45	15	1151.84
end	v6	25/04/91 14-48	18	1597.25
	v7	25/04/91 14-52	22	1149.07
	v8	25/04/91 14-56	26	938.50
	v9	25/04/91 14-58	28	774.39
	v10	25/04/91 15-04	34	637.81
	v11	25/04/91 15-18	48	380.28
	v12	25/04/91 15-48	78	138.03
	v13	25/04/91 16-48	138	40.96
	v14	25/04/91 19-00	150	10.03
	v15	25/04/91 20-48	258	8.75
	v16	25/04/91 23-12	382	7.85
	v17	26/04/91 02-58	748	6.53
	v18	26/04/91 15-06	1476	5.91
1 week post	v19	02/05/91 09-30	8340	1.52
2 weeks post	v20	09/05/91 09-15	19795	0.65
start	v21	16/05/91 15-20	30290	1.32
	v22	16/05/91 15-23	30293	369.88
	v23	16/05/91 15-27	30297	6.84
	v24	16/05/91 15-31	30301	878.44
	v25	16/05/91 15-36	30306	1152.73
	v26	16/05/91 15-42	30312	1613.47
end	v27	16/05/91 15-54	30324	2078.36
	v28	16/05/91 15-57	30327	1728.84
	v29	16/05/91 16-00	30330	1017.35
	v30	16/05/91 16-06	30336	1006.49
	v31	16/05/91 16-10	30340	938.80
	v32	16/05/91 16-15	30345	724.47
	v33	16/05/91 16-25	30355	518.46
	v34	16/05/91 16-58	30388	134.89
	v35	16/05/91 18-01	30451	49.72
	v36	16/05/91 20-00	30570	32.04
	v37	16/05/91 22-00	30690	27.39

Table 38. Platinum in plasma ultrafiltrate for case number 116799.

Start and end of infusion	Sample number	Date and time	Time (minutes)	Pt (ng ml^{-1})
	v38	17/05/91 00-04	30814	25.46
	v39	17/05/91 04-00	31050	18.11
	v40	17/05/91 16-43	31813	15.51
1 week post	v41	23/05/91 09-17	40007	4.51
2 weeks post	v42	30/05/91 09-30	50100	2.81
3 weeks post	v43	06/06/91 12-00	60330	1.13
	v44	10/06/91 09-30	65940	1.04
6 weeks post	v45	26/06/91 10-00	89010	1.49

Table 38(cont.). Platinum in plasma ultrafiltrate for case number 116799.

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