PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 6.

Anticancer agents: qualitative and quantitative aspects

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Introduction

Typically, regulation of human exposure to carcinogenic compounds is based mainly on qualitative considerations for compounds that cause cancer in experimental animals. This approach is based on the old paradigm of using animal models to understand human physiology and pathology; in the regulatory setting, no alternatives to this paradigm have been available specifically in relation to cancer (Maronpot et al., 2004).

Quantitative aspects of carcinogenesis, including estimates of carcinogenic potency in animals and in humans, would have both regulatory and scientific implications. For animals, a systematic assessment of potency based on bioassay data has been generated from values of TD_{50}, i.e. the chronic dose rate (in mg/kg body weight [bw]/day) that is estimated to reduce the proportion of tumour-free animals by 50% (Gold et al., 2005). For humans, exposures to ionizing radiation, occupational carcinogens, and tobacco smoke have been the primary sources of quantitative data on cancer risks, including considerations of dose, duration of exposure, and latency period (i.e. time from exposure to occurrence of cancer) (Breslow and Day, 1987; Moolgavkar et al., 1999; Brenner et al., 2003; Pierce and Vaeth, 2003; Preston et al., 2003, 2004; IARC, 2004). However, because of the complexities of these various human exposures, comparisons of potency assessments with data from animals have been hampered.

An additional source of quantitative data on cancer risks is the group of patients who are survivors of first primary cancers after treatment with anticancer therapy and who are monitored for treatment-related risks of second primary cancers (Travis, 2006; Travis et al., 2006). Active research on second cancers has been carried out since the 1980s, but because of the increasing numbers of patients enrolled and the extended periods of follow-up, the more recent studies provide the most comprehensive evidence on the magnitude of the effects

Table 6.1. Tumour sites and histological types of cancer induced in humans and in rodents after exposure to anticancer agents

Agent	Tumour sites and histological types		
	Humans	**Rats**	**Mice**
Cyclophosphamide	AML Bladder cancer	Lymphoma Leukaemia Mammary adenoma Transitional cell carcinoma of the bladder Neurogenic sarcoma	Lymphoma Acute lymphocytic leukaemia Mammary carcinoma Lung cancer Liver cancer
Chlorambucil	AML	Lymphoma Myeloid leukaemia Mammary carcinoma	Lymphoma Myeloid leukaemia
Melphalan	AML	Retroperitoneal sarcoma	Lymphoma
Thiotepa	Leukaemia	Lymphocytic leukaemia Uterine sarcoma Squamous cell cancers of the skin and the ear canal	Lymphoma Lymphocytic leukaemia

AML, acute myeloid leukaemia.
Source: Compiled from IARC (2012).

(Hijiya et al., 2007; Hodgson et al., 2007; Maule et al., 2007; Hemminki et al., 2008; Swerdlow et al., 2011). Although these studies present valuable data on many exposure-related aspects, relevant treatments are seldom based on single agents or single modalities, and individual carcinogens can rarely be singled out. Nevertheless, striking new data from these studies show increased risks of almost all site-specific cancers that emerge during the follow-up period. Such data challenge the "canonical" site-specificity of carcinogenesis. In their review of human carcinogens, Cogliano et al. indicate that no agents classified as *carcinogenic to humans* (Group 1) are identified as causing prostate cancer (Cogliano et al., 2011). However, some evidence is available. The risk of prostate cancer is significantly increased in survivors of non-Hodgkin lymphoma after chemotherapy, for those diagnosed at age 40–49 years (Hemminki et al., 2008). Anticancer agents are also used in some other cases, such as for certain autoimmune diseases, but even if a single anticancer agent is given, other medication and the inherent cancer risk of some autoimmune conditions may limit the applicability of the results.

In this chapter, data on anticancer agents from Volume 100A of the *IARC Monographs* are used to make qualitative comparisons between cancers induced in humans and in experimental animals, with reference to the possible underlying mechanisms. Furthermore, quantitative comparisons of carcinogens with respect to potency in humans and in experimental animals are discussed. This review is limited to anticancer agents for which the evidence of carcinogenicity was considered to be *sufficient* both in humans and in experimental animals: cyclophosphamide, chlorambucil, melphalan, and thiotepa (IARC, 2012). Of the anticancer agents included in Volume 100A, the current selection does not include busulfan, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU), treosulfan, and some mixtures of anticancer agents for which evidence is *sufficient* in humans but evidence is *limited* or lacking in animals.

Therapeutic applications and trends in use

The four anticancer agents discussed here were first used in clinical practice in the 1960s, but since then the clinical indications have been narrowed and their therapeutic use has declined, with the possible exception of cyclophosphamide.

Cyclophosphamide may be used alone for the treatment of several types of cancer, but most often it is administered in combination with other drugs. Diseases for which cyclophosphamide is the recognized treatment include breast cancer,

Table 6.2. Values of TD_{50} (the chronic dose rate estimated to reduce the proportion of tumour-free animals by 50%) for anticancer agents in rodents

Agent	TD_{50} (mg/kg bw/day)			
	All tumours combined		Haematopoietic malignancies	
	Rats	Mice	Rats	Mice
Cyclophosphamide	2.2[a]	2.8	3.4[b]	7.9
Chlorambucil	0.7	0.1	1.6	0.6
Melphalan	0.6	0.1	0.9	0.5
Thiotepa	0.04	0.07	0.2	0.2

bw, body weight.
[a] Bladder tumours in rats: TD_{50} = 21 mg/kg bw/day (Gold et al., 1987).
[b] From Gold et al. (1987).
Source: Adapted with permission from Kaldor et al. (1988), copyright 1988, with permission from Elsevier.

lymphoma, leukaemia, sarcoma, and ovarian cancer. Cyclophosphamide is also used for treatment of diseases other than cancer, such as nephrotic syndrome and many autoimmune diseases, including Wegener granulomatosis, rheumatoid arthritis, lupus erythematosus, mycosis fungoides, and several forms of vasculitis.

The current clinical use for chlorambucil mainly involves treatment of chronic lymphocytic leukaemia. Chlorambucil may also be used for treatment of non-Hodgkin lymphoma, Waldenström macroglobulinaemia, polycythaemia vera, trophoblastic neoplasms, and ovarian cancer. Chlorambucil has also been applied as an immunosuppressive drug for various autoimmune and inflammatory conditions.

The use of melphalan has declined for treatment of most cancers, but since about 2000 it has been given in high doses to patients with myeloma in combination with autologous stem cell transplantation.

Thiotepa has previously been used in the palliation of a wide variety of neoplastic diseases. It may still be prescribed in intravesical chemotherapy for bladder cancer.

Tumour sites

Tumour sites and histological types of cancer induced in humans and in rodents by the four anticancer drugs are listed in Table 6.1. In humans, cyclophosphamide causes acute myeloid leukaemia and bladder cancer of undefined histology. Lymphoma, leukaemia, and mammary carcinoma have been detected in rats and mice after administration of cyclophosphamide. In rats, transitional cell carcinoma of the bladder and neurogenic sarcoma have been reported. In mice, cancers of the lung and liver have been detected. Chlorambucil causes acute myeloid leukaemia in humans. Lymphoma and leukaemia have been detected in rats and mice, as well as mammary carcinoma in rats. Melphalan causes acute myeloid leukaemia in humans, retroperitoneal sarcoma in rats, and lymphoma in mice. Thiotepa causes leukaemia in humans and lymphocytic leukaemia in rats and mice. It has been reported to induce uterine sarcoma and squamous cell cancers of the skin and the ear canal in rats and lymphoma in mice.

Carcinogenic potency

Gold et al. have systematically analysed the carcinogenic potency of compounds tested in animal experiments in the context of the United States National Toxicology Program (Gold et al., 1987, 2005). These data were used by Kaldor et al. to quantify the carcinogenic potency of anticancer agents (Kaldor et al., 1988). With respect to potency, it should be noted that low daily doses producing cancer (i.e. low TD_{50} values) indicate high carcinogenic potency.

Data on rats and mice from Kaldor et al. (1988) are collected in Table 6.2. For ease of analysis, results for male and female rodents were averaged. When information was lacking in the paper by Kaldor et al., data were taken from other sources, as indicated. The TD_{50} values for all tumours combined are lower than those for haematopoietic malignancies. According to these data, thiotepa is the most potent carcinogen, with TD_{50} values of 0.04 mg/kg bw/day in rats and 0.07 mg/kg bw/day in mice for all tumours combined. Chlorambucil and melphalan

Table 6.3. Estimated carcinogenic potency (10-year cumulative incidence [%] divided by total dose in grams) in humans of anticancer agents

Agent	Leukaemia			Bladder cancer
	From Kaldor et al. (1988)	Calculated[a]		
		Low dose	High dose	
Cyclophosphamide	0.28	–	0.04	0.02[b], 0.1[c]
Chlorambucil	4.2, 1.8	16.5	1.4	–
Melphalan	18.7, 3.3	14.1	11.5	–
Thiotepa	–	55.3	3.2	–

[a] Calculated from Kaldor et al. (1990).
[b] Calculated from Kaldor et al. (1995).
[c] Calculated from Travis et al. (1995).

are equally potent as carcinogens, whereas cyclophosphamide is weaker by approximately an order of magnitude. After treatment of rats with cyclophosphamide, bladder cancer was detected with a TD_{50} of 21 mg/kg bw/day, i.e. an order of magnitude lower than the value for all tumours combined (Gold et al., 1987).

The measure of potency used by Kaldor et al. was the 10-year cumulative incidence of leukaemia (a percentage) divided by the total administered dose in grams; thus, a large number indicates high potency (Kaldor et al., 1988). These data are shown in Table 6.3. Information was lacking for thiotepa, but of the remaining compounds melphalan was the most potent, with values of 18.7 and 3.3 from two separate studies. Chlorambucil showed an intermediate potency, which was an order of magnitude higher than that of cyclophosphamide.

More recent data were added to Table 6.3 from studies in which the anticancer agent was the principal drug used and no radiotherapy was applied. Kaldor et al. published a multinational study of secondary leukaemias in women after treatment for primary ovarian cancer (Kaldor et al., 1990). The potency according to Kaldor et al. was calculated from the cumulative baseline incidence of leukaemia in Sweden of 0.2 per 10 years, multiplied by the relative risk given in the relevant paper; the product was then divided by the median doses cited for the low dose and the high dose (Kaldor et al., 1988). The low and high doses differed widely, and the calculated potency values were clearly higher for the low dose than for the high dose. Thiotepa and melphalan were the most potent drugs, followed by chlorambucil and the much weaker cyclophosphamide. The potency of cyclophosphamide to induce bladder cancer was also calculated, according to the data from two studies; in the first study, bladder cancer was diagnosed in women after treatment for ovarian cancer (Kaldor et al., 1995), and in the second study, bladder cancer was diagnosed in survivors of non-Hodgkin lymphoma (Travis et al., 1995). The use of a cumulative baseline incidence of bladder cancer in women of 0.4 per 10 years for the data of the first study and a sex-adjusted incidence of 0.9 per 10 years for the second study resulted in potency values of 0.02 and 0.1, respectively (Table 6.3). Thus, the potency of cyclophosphamide determined in relation to bladder cancer was lower than its potency in the haematopoietic system. A similar outcome was evident in the rodent studies (Table 6.2).

Mechanisms of action

Cyclophosphamide is activated through a cytochrome P450-mediated reaction to yield phosphoramide mustard and acrolein, both of which can bind to DNA. Phosphoramide mustard undergoes rapid dephosphoramidation, which in neutral in vitro conditions proceeds with a half-life of 8 minutes, resulting in the formation of nornitrogen mustard (Hemminki, 1985). Because most of the metabolic activation of cyclophosphamide takes place in the liver, it seems likely that a considerable proportion of DNA binding in peripheral tissues is in fact mediated by nornitrogen mustard (Hemminki, 1985). As summarized in Volume 100A of the *IARC Monographs*, cyclophosphamide has several endpoints indicative of genotoxic effects

in humans, including DNA damage as measured by the comet assay, mutations at the *HPRT* locus, and sister chromatid exchange. Historically, cyclophosphamide has been included in several genetic structure–activity studies (Vogel et al., 1996, 1998).

Chlorambucil and melphalan are direct-acting derivatives of nitrogen mustard, and thiotepa is a direct-acting trifunctional derivative of aziridine. These compounds bind to DNA and give a positive response in a wide spectrum of assays for genomic injury, including tests for cytogenetic damage, specifically as indicated by chromosomal aberrations and sister chromatid exchange in patients. These drugs have also been included in several genetic structure–activity studies (Vogel et al., 1996, 1998).

Conclusions

For the anticancer drugs cyclophosphamide, chlorambucil, melphalan, and thiotepa, the data summarized in this chapter show that the target sites for which there is *sufficient evidence* of carcinogenicity are generally similar in rodents and humans, particularly for bladder cancer induced by cyclophosphamide.

Anticancer agents allow unique comparisons of carcinogenic potency among species, because the doses administered to humans and animals are known. Cancer treatment has become increasingly multimodal and involves the use of multiple drugs; this makes it difficult to single out individual agents. Also, survival rates have risen and the probability of detecting second tumours has increased. It is unclear why there is not more research activity to follow up other patient groups who receive anticancer agents, such as patients with autoimmune diseases. The current potency data for four anticancer drugs suggest that the TD_{50} values for rats and mice are reasonably homogeneous and consistent. As a carcinogen, cyclophosphamide was the least potent and thiotepa was the most potent agent in any of the rodent models analysed. In humans, cyclophosphamide was the least potent and thiotepa and melphalan were the most potent compounds to induce secondary cancers.

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