A review of human carcinogens

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 14-21 October 2008.

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ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS
METHYL-CCNU

Methyl-CCNU was considered by previous IARC Working Groups in 1980 and 1987 (IARC, 1981, 1987). Since that time, new data have become available, these have been incorporated into the Monograph, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 13909-09-6
Chem. Abstr. Name: Urea, N-(2-chloroethyl)-N′-(4-methylcyclohexyl)-N-nitroso-
IUPAC Systematic Name: 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea
Synonyms: 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea; methyl-CCNU; N′-(4-methylcyclohexyl)-N-(2-chloroethyl)-N-nitrosourea; semustine
Description: Light yellow powder (NTP, 2005)

1.1.1 Structural and molecular formulae, and relative molecular mass

![Methyl-CCNU Molecular Structure]

\[
\text{C}_{10}\text{H}_{18}\text{ClN}_3\text{O}_2
\]
Relative molecular mass: 247.7

1.2 Use of the agent

1.2.1 Indications

Methyl-CCNU is an alkylating agent used alone or in combination with other chemotherapeutic agents to treat several types of cancers, including primary and metastatic brain tumours, Lewis lung tumour, and L1210 leukaemia. It has also been used to treat cancers of the digestive tract, Hodgkin lymphoma, malignant melanoma, and epidermoid carcinoma of the lung (NCI, 1979; US National Institutes of Health, 2000; NTP, 2005).

1.2.2 Dosage

Doses varied depending on the type of cancer and body weight of the individual. The typical oral dose was 125–200 mg/m² body surface area, and was repeated every 6 weeks (NTP, 2005). An alternative regimen was reported to be 200–225 mg/m² orally every 6–8 weeks (NCI, 1979).

Methyl-CCNU was available as 10, 50, and 100 mg capsules (NCI, 1979).
1.2.3 Trends in use

Methyl-CCNU was used in investigational studies in the 1960s and 1970s; it has never been approved as an antineoplastic drug, and is not listed in any of the standard pharmaceutical references and sources. However, some recently published articles indicate that it has been used in the People’s Republic of China to treat various haematopoietic malignancies (Jia et al., 2006; Zhang et al., 2007; Guo et al., 2008).

2. Cancer in Humans

The previous evaluation was based on five studies from the same set of patients described below. No new data were available to the Working Group.

Adjuvant treatment with methyl-CCNU was evaluated in 3633 patients with gastrointestinal cancer treated in nine randomized trials. Among 2067 patients treated with methyl-CCNU, 14 cases of acute myeloid leukaemia occurred, whereas one occurred among 1566 patients treated with other therapies (relative risk, 12.4; 95% confidence interval: 1.7–250). Cumulative (actuarial) risk was 4% at 6 years, and was not affected by concomitant radiotherapy or immunotherapy (Boice et al., 1983). A subsequent report described a strong dose–response relationship, adjusted for survival time, giving a relative risk of almost 40 among patients who had received the highest dose (Boice et al., 1986).

3. Cancer in Experimental Animals

Data on methyl-CCNU were included in a report in which a large number of cancer chemotherapeutic agents were tested for carcinogenicity by intraperitoneal injection in male Sprague-Dawley rats, and male Swiss mice. In rats injected with methyl-CCNU three times weekly for 6 months, the incidence of peritoneal sarcoma increased, and total tumour incidence was reported to increase 1.5-fold over that in controls at 18 months. No increase in tumour incidence was observed in mice (Weisburger, 1977). Intravenous administration of methyl-CCNU to rats was also reported to clearly induce lung tumours (Habs & Schmähl, 1984).

See Table 3.1

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

In humans, following oral administration, methyl-CCNU is well absorbed from the gastrointestinal tract, undergoes rapid chemical decomposition and oxidative metabolism, and is rapidly distributed throughout the body (Sweetman, 2005). Following the oral administration of radiolabelled methyl-CCNU to cancer patients, radioactivity is detected in plasma within 10 minutes, with peak plasma levels attained within 3–6 hours. Radioactivity is slowly eliminated from the plasma, with a half-life of 36 hours reported for the chloroethyl moiety, and two half-lives for the cyclohexyl moiety: an initial half-life of 24 hours, and a subsequent half-life of 72 hours. Approximately 60% of the administered radioactivity is excreted in the urine within 48 hours (Sponzo et al., 1973).

In addition to chemical degradation, methyl-CCNU is metabolized by the cytochrome P450 (CYP) mono-oxygenase system on the cyclohexyl ring carbons, and the 2-chloroethyl side-chain (Reed, 1994). CYP-dependent formation of alkylating metabolites from methyl-CCNU has been observed in studies in vitro with rat liver microsomes. The alkylating metabolites bind covalently to DNA and protein (Kramer, 1989; Reed, 1994), and increase mutagenicity in bacteria (Franza et al., 1980). Methyl-CCNU is also metabolized to carbamylating species, but subsequent carbamoylation reactions are not
4.2 Genotoxic effects

Methyl-CCNU is a bifunctional antineoplastic agent that undergoes spontaneous chemical decomposition yielding electrophilic compounds. These induce alkylation and carbamoylation of cellular macromolecules, including DNA and protein (Kramer et al., 1986; Kramer, 1989; Reed, 1994). As with other chloroethylnitrosoureas, the majority of the alkylation reactions occur at the $N^7$ position of guanine, but the critical reaction leading to cytotoxicity is reported to involve alkylation of the $O^6$ of guanine, which leads to G–C cross-links in DNA (Chu & Sartorelli, 2007). Carbamoylation of proteins is also believed to contribute to the toxicity of methyl-CCNU, and may contribute to carcinogenesis through inhibition of DNA-repair processes (Kramer, 1989; Reed, 1994).

Methyl-CCNU has been tested for genotoxicity in several short-term assays in vitro and in vivo. The administration of methyl-CCNU in vivo results in DNA adducts in the bone marrow, spleen and colon of treated mice (Wheeler et al., 1983), and in the kidney, liver and lung of treated rats (Kramer et al., 1985, 1986). Large increases in the frequency of micronuclei are also seen in the bone-marrow erythrocytes of mice treated with methyl-CCNU (Tinwell & Ashby, 1991; Ashby et al., 1993). Methyl-CCNU induces chromosomal aberrations, micronuclei, sister chromatid exchange, and DNA strand breaks in human or rodent cells in vitro (Erickson et al., 1978; Wheeler et al., 1983; Shah et al., 1986; Baumler et al., 1987; Vyas et al., 1988; Tapiero et al., 1989). It also induces mitotic crossing-over in yeast (Ferguson & Turner 1988a, b), and is mutagenic in bacteria (Auletta et al., 1978; Franza et al., 1980; Ashby et al., 1993).

In addition, patients treated with methyl-CCNU (in combination with 5-fluorouracil and vincristine) as cytostatic therapy were found to have increased frequencies of sister chromatid exchange and chromosomal aberrations in their peripheral blood lymphocytes (Gebhart et al., 1980a, b). Myelosuppression has also been reported in patients treated with methyl-CCNU (Young et al., 1973; Breeden et al., 1982).

### Table 3.1 Studies of cancer in experimental animals exposed to methyl-CCNU

<table>
<thead>
<tr>
<th>Species, strain (sex)</th>
<th>Route</th>
<th>Dosing regimen</th>
<th>Incidence of tumours</th>
<th>Significance</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat, Sprague-Dawley (M) 18 mo</td>
<td>i.p.</td>
<td>0, 1.5, 3.0 mg/kg 3 ×/wk for 6 mo 25/group 179 controls</td>
<td>Peritoneal sarcomas: 0/179 (0%), 3/49 (6.1%) Lung: 3/179 (1.1%), 3/49 (6.1%) Mammary gland: 4/179 (2.2%), 3/49 (6.1%) Brain: 2/179 (1.1%), 1/49 (2.0%)</td>
<td>$P &lt; 0.009$</td>
<td>Purity NR Dosage groups were combined for each sex Overall increase in malignant tumours: 1.5-fold (53% vs 34%)</td>
</tr>
<tr>
<td>Rat, Wistar (M) Lifetime</td>
<td>i.v.</td>
<td>0, 19, 38, 75, 150 mg/m² once every 6 wk 30/group 120 controls</td>
<td>Lung: +, [proven evidence according to the authors]</td>
<td></td>
<td>Purity &gt; 99% (for clinical use)</td>
</tr>
</tbody>
</table>

i.p., intraperitoneal; i.v., intravenously; M, male; mo, month or months; NR, not reported; vs, versus; wk, week or weeks
4.3 Mechanisms of carcinogenesis

Acute myeloid leukaemia that develops in patients who have previously been treated with alkylating agents such as methyl-CCNU frequently exhibits distinctive characteristics that allow it to be distinguished from acute myeloid leukaemia induced by other agents (such as topoisomerase II inhibitors) or acute myeloid leukaemia that occurs spontaneously (Pedersen-Bjergaard & Rowley, 1994; Jaffe et al., 2001; Smith et al., 2003; Pedersen-Bjergaard et al., 2006).

One of the hallmarks of leukaemias induced by alkylating agents is that they frequently exhibit a clonal loss of either chromosome 5 or 7 (−5, −7) or a loss of part of the long arm of one of these chromosomes (5q−, 7q−). For example, a deletion within the long arm of chromosome 5 involving the bands q23 to q32 is often seen (Jaffe et al., 2001).

In addition, mutations in TP53 are frequently seen in leukaemias with the −5/5q− karyotype, and mutations involving the AML1 gene as well as mutations in TP53 and RAS are also seen in a subset of leukaemias that exhibit the −7/7q− karyotype (Christiansen et al., 2001, 2005; Pedersen-Bjergaard et al., 2006). These treatment-related acute myeloid leukaemias also frequently exhibit increased methylation of the p15 promoter (Pedersen-Bjergaard et al., 2006). Although methyl-CCNU has not been directly shown to induce losses or deletions affecting chromosomes 5 or 7, this drug has been reported to induce similar types of chromosomal alterations and deletions in a variety of experimental models (see description above), and in the lymphocytes of treated patients (Gebhart et al., 1980a, b). The detection of elevated levels of chromosomal aberrations in the peripheral blood lymphocytes of patients treated with methyl-CCNU is of particular note, as multiple prospective studies have shown that individuals with increased levels of chromosomal aberrations in these cells are at increased risk of developing cancer (Hagmar et al., 1998, 2004; Liou et al., 1999; Smerhovsky et al., 2001; Boffetta et al., 2007).

4.4 Synthesis

Methyl-CCNU is a direct-acting alkylating agent that is carcinogenic via a genotoxic mechanism.

5. Evaluation

There is sufficient evidence in humans for the carcinogenicity of methyl-CCNU. Methyl-CCNU causes acute myeloid leukaemia.

There is limited evidence in experimental animals for the carcinogenicity of methyl-CCNU.

Methyl-CCNU is carcinogenic to humans (Group 1).

References


