in the traumatically injured spinal cord of mammals, including humans. Whereas such agents as methylprednisolone sodium succinate, naloxone, TRH, and ε-ACA must be used promptly to prevent damage during the early, acute phase of injury, clonidine administration can wait until the vital signs have stabilized after spinal shock. Nevertheless, the sooner clonidine is administered the more likely it is to minimize the muscular atrophy and occurrence of other dysfunctions associated with the flaccidity of the acute phase of traumatic injury. Clonidine stimulates the α_2-receptors in the intermedio-lateral cell columns of the spinal cord and in the ventral horn which in turn inhibit γ- and α-motor neurons. The random visceral and somatic afferent inputs into the distal stump of the traumatically injured spinal cord of humans and other animals that cause autonomic dysreflexia (*3*) and spasticity (Fig. 3) are thus blocked by clonidine's tonic stimulation of α_2-adrenergic receptors.

Clonidine has been administered orally to more than 30 humans with spasticity resulting from a traumatic cervical or thoracic transverse myelopathy (*16*). The drug has served to check the potentially dangerous increases in arterial blood pressure during episodes of autonomic dysreflexia (*7*). It has also prevented the spasticity (Fig. 3) of musculature which debilitates most of these patients and frequently retards their rehabilitation. Because of the depressive effect of clonidine on sympathetic preganglionic outflow, this drug must be used with caution.

This new approach to the restoration of function in the traumatically injured CNS of mammals may find use in the immediate or delayed treatment of traumatic injuries to the spinal cord as well as brainstem lesions and cerebrovascular accidents.

N. ERIC NAFTCHI

Laboratory of Biochemical Pharmacology, New York University Medical Center, New York 10016

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13. To record SEP's we placed the cathodes proximal to the anodes. Rectangular pulse stimuli were delivered from a Grass S2 stimulator by way of a stimulus isolation unit. A frequency of 1 Hz, with 1-msec duration, was adjusted to sufficient intensity to produce visible contractions of the lower extremity. Once set, the intensity was not changed for the rest of the experiment. Recordings were made with two vanadium screws inserted through drill holes made into the skull. One was placed in the midline over the sensory cortex for the lower extremities and the second at about 1 cm anterior to the first. A ground electrode, lightly coated with electrolyte gel, was taped over a shaved ear. Potentials were led into a Grass P511B preamplifier and magnified 20,000 times with a bandwidth of 15 to 3.2 Hz. Amplified signals were further processed through a Nicolett Signal Averager. We used a dwell time of 250 msec per point, and 65 to 100 consecutive responses were summed.
14. Carotid arterial blood pressure was measured with a pressure transducer (Statham, P23Db) attached to a four-channel recorder (Dynograph, Beckman Instruments, Inc.). Abdominal aorta blood flow was measured by means of an electromagnetic blood flow transducer and an electromagnetic blood flow meter (Biotronex Laboratory, model BL610), the output of which was connected to the same four-channel recorder.
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Polyamine Depletion Influences Drug-Induced Chromosomal Damage

Abstract. *Polyamines have been implicated in the intracellular stabilization of DNA. Depletion of intracellular polyamines influences the cytotoxicity of 1,3-bis(2-chloroethyl)-1-nitrosourea and* cis*-diamminedichloroplatinum II. By means of the sister chromatid exchange assay, it was found that intracellular polyamine depletion can also alter the induction of chromosomal damage by these cytotoxic agents.*

The polyamines putrescine, spermidine, and spermine have been implicated in the regulation of both normal and neoplastic cell proliferation (*1, 2*). There are several proposed roles for these polycations in cellular metabolism including the stabilization of nuclear DNA (*3*). Polyamines stabilize cell-free DNA to enzymatic degradation (*4*), denaturation by x-rays (*5*), and thermal denaturation (*6*). X-ray diffraction studies suggest that primary and secondary amine groups of spermidine and spermine bind ionically to adjacent phosphate groups on one strand of DNA, and the four-carbon chain stretches across the minor groove of the double helix to form a cross bridge between phosphate groups on opposite strands (*7*). However, on the basis of the theory of counterion condensation, Bloomfield and Wilson postulate that the polyamine-mediated stabilization of DNA is the result of relatively nonspecific electrostatic interactions between polyanionic DNA and the cationic polyamines (*3*). Although the specific interactions of the polyamines and DNA have not been clearly defined, it does appear that they are important in stabilizing DNA structure. Viscoelastometry experiments indicate that there is an alteration in the conformation of DNA or its susceptibility to shear in x-irradiated cells made deficient in polyamines (*8*). Analyses of the structure of Z-DNA (*9, 10*), indicate that spermine is located not only adjacent to the phosphate groups but also adjacent to DNA bases.

The stabilizing effect of polyamines on the structure of DNA, the probable target of many antineoplastic drugs, suggests a possible role for the depletion of polyamines in cancer chemotherapy. Cellular polyamine concentrations can

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be depleted by treatment with α-difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase (ODC), the first and rate-limiting enzyme of the polyamine biosynthetic pathway (*11*). Treatment of rat 9L brain tumor cells in vitro with a noncytotoxic concentration of DFMO (10 m*M*) for 48 hours depresses putrescine and spermidine to less than 5 percent of control levels with spermine remaining essentially unchanged (*12*). Using this DFMO treatment protocol, Hung *et al.* (*13*) demonstrated that polyamine deficiency increases the cytotoxicity of 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU), a nitrosourea generally considered to kill cells through alkylation of DNA followed by the formation of interstrand DNA-DNA cross-links (*14*). This effect was reversed by the addition of putrescine to the culture medium after depletion with DFMO but before treatment with BCNU, thus indicating specificity for polyamine depletion. Hung *et al.* (*13*) postulate that DFMO, by inhibiting polyamine synthesis, destabilizes DNA rendering it more susceptible to alkylation and subsequent interstrand cross-linking by the reactive moiety of BCNU. Recently, we showed that DFMO does not affect the alkylation of DNA by 1-(2-chloroethyl)-3-*trans*-4-methylcyclohexyl-1-nitrosourea (MeCCNU), a nitrosourea that is also an alkylating agent that induces cross-link formation (*15*). The effects of DFMO on interstrand cross-linking, the event considered to be responsible for most MeCCNU- and BCNU-induced cytotoxicity, remains to be determined.

Oredsson *et al.* (*16*) showed that treatment of 9L cells with DFMO decreased the cytotoxicity of *cis*-diamminedichloroplatinum II (*cis*-DDP), a compound that is thought to kill cells by forming DNA-DNA inter- and intrastrand cross-links (*17*). This decreased cytotoxic effect was also reversed by the addition of putrescine. Laurent *et al.* (*18*) have presented data from alkaline elution studies suggesting that the cross-links formed by *cis*-DDP are different from those formed by chloroethylnitrosoureas. The two chlorine atoms, the active groups in *cis*-DDP, are separated by 3.3 Å (*19*), which is similar to the interplanar distance of DNA bases (3.4 Å) (*20*); a number of nucleophilic groups in native DNA are also separated by 3.4 Å (*21*). Therefore, a possible explanation for the DFMO-mediated decrease in *cis*-DDP cytotoxicity is that polyamine deficiency, resulting in an alteration in DNA structure, might change the distance between these nucleophilic groups and thus prevent cross-link formation (*16*).

Thus polyamine deficiency can either increase or decrease drug-induced death of cells, depending on the mechanism of drug action. If these effects are indeed the result of DNA destabilization caused by polyamine deficiency, then changes of an equivalent nature should be detected on a chromosomal level. The sister chromatid exchange assay is a sensitive yet relatively simple method for the measurement of chromosomal damage (*22*); the induction of such exchanges by compounds that damage DNA has been clearly established (*23*). Therefore, to determine the effects of polyamine deficiency on drug-induced chromosomal damage, we have examined the ability of DFMO to modify the induction of sister chromatid exchanges in 9L cells treated with BCNU and *cis*-DDP.

Rat 9L brain tumor cells were seeded and cultured as described (*13*). For the treatment of cells with DFMO or DFMO plus putrescine in combination with either BCNU or *cis*-DDP we used the same procedures as described in the aforementioned cytotoxicity experiments (*13, 16*). The sister chromatid exchange assay of Perry and Wolff (*24*) was used (Tables 1 and 2).

Treatment of cells with DFMO alone did not alter the number of chromatid exchanges, whereas 1 μ*M* BCNU increased the exchanges with respect to control levels (Table 1). However, in cells treated first with DFMO and then with BCNU, the number of exchanges increased approximately twofold over the number in cells treated with BCNU alone. Cells treated with putrescine as well as DFMO before being exposed to BCNU eliminated this increase indicating that, as with cytotoxicity, the DFMO potentiation of BCNU-induced sister chromatid exchanges is due to polyamine deficiency.

Although *cis*-DDP was effective in inducing sister chromatid exchanges in 9L cells, if the cells were treated with DFMO before being exposed to *cis*-DDP the number of exchanges was reduced (Table 2); this effect was eliminated by addition of putrescine. Thus, polyamine deficiency not only decreases *cis*-DDP–induced cytotoxicity, but also decreases *cis*-DDP–induced sister chromatid exchanges.

Polyamine deficiency has been postulated to destabilize DNA and thus alter the interactions between chemotherapeutic agents and DNA. Our experiments demonstrate that DFMO-mediated polyamine deficiency also modifies the induction of chromosomal damage by BCNU and *cis*-DDP, as measured by sister chromatid exchange assay, and thus lends support to this concept. Inhibitors of polyamine biosynthesis are being investigated as potential biological response modifiers for use in cancer chemotherapy, and it appears that their use may either increase or decrease the intracellular action of a chemotherapeutic agent, depending on the agent's mechanism of action. Therefore, optimal combinations of and administration sched-

Table 1. The effects of DFMO on sister chromatid exchanges (SCE's) induced by BCNU. Cells were incubated with 10 m*M* DFMO for 72 hours and then with 1 μ*M* BCNU for 1 hour. In the putrescine reversal experiments the above protocol was followed except that after 48 hours of incubation with DFMO, putrescine was added to achieve a final concentration of 1 m*M*. After drug treatment, the cells were rinsed and fresh medium was added containing 10 μ*M* bromodeoxyuridine. Cells were then allowed to replicate for two cell cycles and collected for SCE analysis (*24*). Values represent the means ± standard deviation. Numbers in parentheses indicate the number of metaphases scored.

Treatment	SCE's per metaphase
Control	11.6 ± 3.1 (40)
DFMO	14.7 ± 4.8 (20)
Putrescine	13.1 ± 4.2 (20)
DFMO plus putrescine	14.1 ± 3.7 (40)
BCNU (1 μ*M*)	27.2 ± 6.1 (40)
Putrescine plus BCNU (1 μ*M*)	26.0 ± 4.4 (20)
DFMO plus BCNU (1μ*M*)	51.4 ± 11.0 (41)
DFMO plus putrescine plus BCNU (1 μ*M*)	26.1 ± 4.4 (40)

Table 2. The effects of DFMO on sister chromatid exchanges (SCE's) induced by *cis*-DDP. Cells were incubated with 10 m*M* DFMO for 72 hours and then with 0.5 μ*M* *cis*-DDP for 1 hour. In the putrescine reversal experiments the above protocol was followed except that after 48 hours of incubation with DFMO, putrescine was added to achieve a final concentration of 1 m*M*. After drug treatment, the cells were rinsed and fresh medium was added containing 10 μ*M* bromodeoxyuridine. The cells were then allowed to replicate for two cell cycles and collected for sister chromatid exchange analysis (*24*). Values represent the means ± standard deviation. Numbers in parentheses indicate the number of metaphases scored.

Treatment	SCE's per metaphase
Control	12.5 ± 3.3 (20)
cis-DDP (0.5 μ*M*)	56.5 ± 12.4 (40)
Putrescine plus *cis*-DDP (0.5 μ*M*)	54.3 ± 12.6 (20)
DFMO plus *cis*-DDP (0.5 μ*M*)	29.7 ± 7.0 (41)
DFMO plus putrescine plus *cis*-DDP (0.5 μ*M*)	50.0 ± 11.1 (21)

ules for antineoplastic drugs and polyamine biosynthesis inhibitors should be determined in the laboratory before the compounds are used in the clinical setting.

PHILIP J. TOFILON
STINA M. OREDSSON
Brain Tumor Research Center of the Department of Neurological Surgery, School of Medicine, University of California, San Francisco 94143
DENNIS F. DEEN
Brain Tumor Research Center of the Department of Neurological Surgery and *Department of Radiation Oncology, School of Medicine, University of California, San Francisco*
LAURENCE J. MARTON
Brain Tumor Research Center of the Department of Neurological Surgery, and *Department of Laboratory Medicine, School of Medicine, University of California, San Francisco*

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Human β-Globin Gene Sequences Injected into Mouse Eggs, Retained in Adults, and Transmitted to Progeny

Abstract. *Foreign gene sequences were retained in two adult mice (out of 62 analyzed) from fertilized eggs injected with a recombinant plasmid containing the human β-globin genomic region and the herpes simplex viral thymidine kinase gene. The intact human and viral genes were found in DNA of one of the animals and, in the other, at least part of the human globin gene was present. The latter individual transmitted these sequences to its progeny in a Mendelian ratio. Thus, human DNA may be incorporated into the germ line of mice for in vivo studies of regulation of gene expression in development, genetic diseases, and malignancy.*

In a recent study, we demonstrated that human and viral genes introduced into fertilized mouse eggs by microinjection of recombinant DNA into a pronucleus could later be found intact in DNA prepared from fetuses near the end of their prenatal development (*1*). In the present report, eggs injected with the same genes were allowed to develop to adulthood. Persistent foreign genetic material was detected in some adults, and biological evidence for integration in host DNA was obtained through Mendelian transmission of human β-globin sequences to progeny.

Approximately 3000 copies of the bacterial plasmid PtkHβ1 were injected into the male pronucleus of (C3H × DBA/2)F_1 fertilized mouse eggs, as previously described (*1*). The plasmid consists of a pBR322 vector with a 7.6-kb Hind III insert carrying the human β-globin (HβG) genomic region and a 3.6-kb Bam HI fragment containing the thymidine kinase (tk) gene of herpes simplex virus (HSV) (*2, 3*). Of 394 eggs injected, 304 survived the injection and were surgically transferred to the oviducts of pseudopregnant Icr random-bred females. At 8 to 10 weeks of age, partial splenectomy was performed on 62 survivors, and the DNA was extracted (*4, 5*).

Some of the DNA was digested with the restriction endonuclease Pst I and tested by the Southern procedure (*6–8*) for hybridization with the ^{32}P-labeled 7.6-kb globin gene insert. The DNA's of two of the spleens hybridized with the HβG probe and therefore contained some gene sequences of human origin. One of the positive experimental animals (designated Exp. 1), a female, had the diagnostic 4.4-kb Pst I fragment that spans the entire coding region and also the neighboring 3.9-kb fragment (*9*), thereby indicating retention of the entire intact human globin insert, as seen by comparison with a control digest of the injected plasmid (Fig. 1A). When the animal was ultimately autopsied, after breeding, the DNA digests from its five other tested organs (liver, kidney, heart, lung, brain) showed the same result as the spleen, with approximately two copies of the HβG per cell in all tissues. The second experimental animal with HβG sequences (Exp. 2), a male, had a higher molecular weight band of 13 kb, which hybridized with the 7.6-kb human gene insert. This band was present in all seven organs eventually tested, at an estimated copy number of one per cell (Fig. 1C). In subsequent experiments aimed at identifying these sequences, Bam HI and Eco RI digests were examined for hybridization with the same HβG probe (Fig. 2A, lanes F_1 a′ and F_1 a″); they did not display the bands expected if the coding region were present. In addition, no hybridization with an HβG complementary DNA (cDNA) probe was observed (data not shown). The 13-kb band was, however, seen when the probe was a 3.6-kb Eco RI fragment including most of the 3′ end of the genomic region. Therefore, the HβG sequences in this second animal included at least some of the 3′ sequences flanking the coding region of the human β-globin gene.

DNA's from the same spleen samples were also analyzed for presence of HSV tk gene and pBR322 sequences by digestion with Bam HI and hybridization with ^{32}P-labeled Ptk. This probe represents the vector after insertion of the HSV tk gene into the Bam HI site of pBR322 (*3*) and before introduction of the HβG fragment. The animal (Exp. 1) that was positive for the intact HβG gene also exhibited Ptk-hybridizing sequences, at approximately two copies per cell, in all five organs eventually analyzed (Fig. 1B). The other HβG-positive individual, Exp.

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