experiments was 945 fmol per mg of protein in L-RGB2Zem-1 membranes and 454 fmol per mg of protein in rat striatal membranes. The binding of [^{3}H]spiperone to membranes from L-RGB2Zem-1 cells was inhibited by a number of drugs and the resulting K_i values closely matches those obtained using striatal membranes (Fig. 3*b*, Table 1). The D_2 antagonists (+)butaclamol and haloperidol were the most potent inhibitors, followed by sulpiride. The D_1 dopamine antagonist SCH 23390 and the 5-HT_2 antagonist ketanserin were much less potent at blocking [^{3}H]spiperone binding. The binding seemed to be stereoselective as (+)butaclamol was much more potent than (−)butaclamol at inhibiting binding. In these experiments the absolute affinities of dopaminergic antagonists and the rank order of potency of the drugs (spiperone > (+)butaclamol > haloperidol > sulpiride ≫ (−)butaclamol) agree closely with previously published values for the D_2 dopamine receptor[2].

The physiological effects of stimulation of D_2 dopamine receptors seem to be mediated by G_i[6]. Inhibition of agonist binding to D_2 dopamine receptors by GTP is thought to be due to GTP-induced dissociation of a receptor-G_i complex which has a high affinity for agonist[21,22]. Binding of [^{3}H]spiperone was inhibited by the agonist dopamine with a K_i of 17 μM and a Hill coefficient of 1 in L-RGB2Zem-1 membrane. This dopamine binding was not responsive to the addition of GTP. This finding is consistent, however, with the reported lack of G_i in L cells[23]. The pharmacological data presented here prove that the binding profile of the D_2 dopamine receptor is found in Ltk$^-$ cells expressing the RGB-2 cDNA.

When transfected into eukaryotic cells, the RGB-2 cDNA directs the expression of a D_2 dopamine-binding protein. As the mRNA corresponding to this cDNA is localized in tissues where the D_2 dopamine receptor is known to be present and as this mRNA codes for a protein that has all the expected characteristics of a G-protein-coupled receptor, we conclude that RGB-2 is a clone for the rat D_2 dopamine receptor. The successful cloning of the D_2 dopamine receptor will provide new tools to study the regulation and function of this receptor.

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A novel cancer therapy using a Mössbauer-isotope compound

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Cancer radiotherapy uses high doses of ionizing radiation (1–10^2 Gy; 10^2–10^4 rad) because only a small fraction of the absorbed dose leads to lethal double-strand breaks in DNA. These breaks are more efficiently produced by Auger electrons (1–10 eV nm^{-1}) generated in proximity to the DNA. The energy of these electrons (on average 21 electrons for the decay of ^{125}I) is dissipated within 10–100 nm of the Auger event and produces multiple double-strand DNA breaks[1,2]. A single Auger event can be lethal to a cell and is comparable to more than 10^5 photon absorption events in conventional radiotherapy[3,4]. We now report that ^{57}Fe(III)·bleomycin, administered to malignant cells *in vitro* and *in vivo* and irradiated with resonant Mössbauer gamma rays (14.4 keV), causes ablation of the malignant cells, presumably by Auger cascade, with extremely small radiation doses—about 10^{-5} Gy. As a basis for comparison, about 5 Gy is necessary to achieve a similar effect with conventional radiotherapy[5].

Bleomycin is one of many flat ring-shaped molecules that bind strongly to DNA by intercalating (inserting) between base pairs. The biologically active form of the drug is Fe(III)·bleomycin. In this therapy (see the legend to Table 1 for the overall scheme) the photon-absorbing atom must be a Mössbauer isotope. Thus, we have prepared and used ^{57}Fe(III)·bleomycin as our Mössbauer-isotope pharmaceutical.

After addition of the ^{57}Fe(III)·bleomycin to the target cells, the cells were irradiated with highly monochromatic 14.4 keV gamma rays from excited-state ^{57}Fe nuclei (a Mössbauer isotope). In Mössbauer absorption, the radiation source is a collection of excited nuclei bound to a crystal lattice which emit highly monochromatic gamma rays as they decay to the ground state. The sample (absorber) consists of ground-state nuclei of the same isotope bound to a crystal lattice, which may or may not be the same lattice as the source lattice. In this case, ^{57}Co nuclei decay by electron capture to excited ^{57}Fe nuclei (the source nuclei), which emit highly monochromatic gamma-rays as they decay to the ground state: 137 keV, 123 keV and 14.4 keV. The absorbing nuclei are stable ground-state ^{57}Fe nuclei. When ^{57}Fe in the source and absorbing matrices is tightly bound, and when the source and absorbing matrices are the same, resonant absorption occurs (14.4 keV) because there is no recoil energy loss or gain by ^{57}Fe in either medium, and because the chemical environments are the same. However, if the energy states of the nuclei in the absorbing crystal are even slightly altered (by a different chemical environment or by an applied field, for example), the gamma rays will not be absorbed unless a Doppler velocity is applied to the source to compensate for the slightly

Table 1 The effect of treatment with drug $^{57}Fe(III)$·bleomycin and Mössbauer radiation on cell line HTB26 (human breast cancer tissue)

Experiment	Proliferation time (h)	Cell proliferation relative to no-treatment cells: No treatment	Drug alone	MIRAGE*
1	192	100	79	32
2	193	100	71	24
3	168	100	82	0
4	168	100	67	17
5	144	100	88	25
6	120	100	92	33
7	120	100	100	20

$^{57}Fe(III)$·bleomycin was prepared by dissolving ^{57}Fe metal (NEN) in concentrated HCl and neutralizing the solution with sodium hydroxide until $Fe(OH)_3$ just began to precipitate. Blenoxane (Bristol Myers) was added to the mixture (two Fe atoms per belomycin). The *p*H of the yellow solution was adjusted to 7.4. The cells were grown in medium (Dulbecco's modified Eagles medium with 10% fetal bovine serum, 50 $\mu g\,ml^{-1}$ streptomycin, 100 $\mu g\,ml^{-1}$ vancomycin and 2 nM glutamine) in T25 flasks until a monolayer was obtained. Cells were treated in groups: (1) No-treatment cells—the monolayer of each flask was washed twice with iron-free medium (special order from Gibco); no drug or radiation was administered. (2) Drug alone—the monolayer of each flask was washed twice with iron-free medium; 50 μl drug $^{57}Fe(III)$·bleomycin (2.3×10^{-4} M) was administered without radiation; the final concentration of $^{57}Fe(III)$·bleomycin was 3.8 μM. (3) MIRAGE—the monolayer of each flask was washed twice with iron-free medium; 50 μl drug $^{57}Fe(III)$·bleomycin was administered and cells were irradiated for 65 min (5 h in experiment 3). After the time of the experiment had elapsed, the drug was removed by washing the monolayer twice with iron-free growth medium and once with phosphate-buffered saline. The cells were trypsinized with 5% trypsin EDTA and 10^5 cells from each experiment were passed into a new T25 flask containing a growth medium (counting was performed using methylene blue stain and a haemocytometer). The cells were grown as a monolayer for a period of time (120–192 h), after which they were trypsinized and counted a second time. The source (^{57}Co in a rhodium matrix) was driven at +1.5 mm s^{-1}, which is resonant with the Mössbauer absorption peak of this pharmaceutical[18], by an Austin Science S-700 constant-velocity drive module in combination with an Austin Science K4 linear motor. The radiation dose was 8.3×10^{-5} Gy h^{-1} (total) and 0.93×10^{-5} Gy h^{-1} for the 14.4 keV gamma ray. The overall scheme is as follows. (1) Bleomycin is derivatized with $^{57}Fe^{3+}$ to give the drug, $^{57}Fe(III)$·bleomycin. (2) The drug is administered to the target cells. The drug binds strongly to DNA. One drug molecule intercalates about every 8–10 base pairs. (3) The target cells are irradiated with 14.4 keV photons (about 10^{-5} Gy). (4) When an ^{57}Fe atom absorbs a 14.4 keV photon, the nucleus is transformed into an unstable excited state. About 90% of excited ^{57}Fe atoms decay by internal conversion (a nuclear rearrangement), followed by an Auger cascade (emission of many electrons). A molecular 'explosion' occurs in the vicinity because firstly the energy of the Auger electrons is dissipated within 10–100 nm, and secondly the cluster of positively charged atoms is blown apart by Coulombic repulsion as the highly charged Fe atom ionizes nearby atoms. (5) The Auger cascade and molecular explosion produce multiple double-strand breaks in the DNA.

* Velocity, 1.5 mm s^{-1}.

different nuclear energy levels in the two media. Extremely sharp lines that correspond to the natural lifetime of the excited state can be observed.

Of importance here is the fate of the excited ^{57}Fe absorber nuclei, which are tightly bound near the DNA. De-excitation occurs predominantly by internal conversion (a nuclear rearrangement) followed by an Auger cascade. In addition to the damage caused by the Auger electrons, the positive charge on the cascading ^{57}Fe atom (Fe^{+11} for eight Auger electrons) ionizes nearby atoms and causes a 'molecular explosion' because of the coulombic repulsion among this cluster of positively charged atoms. It is not surprising that multiple double-strand breaks result when these events occur near DNA.

Resonant photon absorption by a Mössbauer isotope is much more probable than resonant photon absorption by other atoms in tissue, because the resonant-absorption cross-section for Mössbauer absorption (a nuclear excitation) is typically eight orders of magnitude greater than the total absorption cross-section of tissue. (The resonant-absorption cross-section, 2.2×10^{-17} cm^2 for Fe, should not be confused with the photoelectric cross-section, 5.5×10^{-21} cm for Fe, which is typically three orders of magnitude less and requires radiation of a different multipolarity[6].) Assuming one intercalated molecule (and one ^{57}Fe) per eight base pairs[7–9], 10^{10} base pairs per cell genome, and that one Mössbauer absorption per cell will be a lethal event, we calculate a required photon fluence of 3.8×10^7 photons cm^{-2}. Assuming that 60% of the photons are absorbed in 1 cm, we calculate a required dose (14.4 keV) of 5×10^{-5} Gy.

The effects of 1×10^{-5} Gy doses of Mössbauer radiation on a human breast cancer cell line, HTB26, are shown in Table 1. The proliferation per cent in cell numbers was calculated as (final cell count of experiment)/(final cell count of No-treatment) × 100%. The ratio of the means of proliferation of the 'drug-alone' group (no Mössbauer radiation) and the MIRAGE (Mössbauer isotopic resonant absorption of gamma emission) group ($^{57}Fe(III)$·bleomycin with Mössbauer radiation) was 4.0; that is, the MIRAGE therapy reduced the cell proliferation by a factor of 4. The difference of the means of cell proliferation of the drug-alone group and the MIRAGE group was 62% ($P<0.005$). Similar results were obtained for human lung cancer cell line A549 and human breast cancer cell line MCF7.

The results of the treatment of tumour-bearing C3H mice with drug alone ($^{57}Fe(III)$·bleomycin, no Mössbauer radiation) and with MIRAGE therapy (drug in combination with Mössbauer radiation) are given in Tables 2 and 3 respectively. The inhibition of growth index was calculated as 87% using the formula

$$\left(1-\frac{T_n/T_0}{C_n/C_0}\right)\times100\%$$

Where T_n is the mean tumour volume of the MIRAGE group on day 17, T_0 is the initial mean tumour volume of the MIRAGE group, C_n is the mean tumour volume of the drug-alone group on day 17, and C_0 is the initial mean tumour volume of the drug-alone group. The mean final:initial tumour volume ratio of the drug-alone group is 14. The mean final:initial tumour volume ratio of the MIRAGE group is 1.9. Thus, the average tumour was 7.4 times larger in the drug-alone group than in the MIRAGE group (14/1.9). The difference of the means of the ratios of the final:initial tumour volume of each mouse of the MIRAGE group and the drug-alone group is 12.1 ($P<0.005$). The MIRAGE group showed no weight change, whereas the drug-alone group demonstrated a significant increase in weight attributable to the increase in tumour bulk.

We have demonstrated a statistically significant reduction in cell proliferation in cell lines HTB26, A549 and MCF7, and in tumour bulk of C3H mice treated with intercalated $^{57}Fe(III)$·bleomycin and about 10^{-5} Gy of 14.4 keV radiation, which is of no consequence biologically. It is important to note that the Mössbauer isotope-carrying compound need not be toxic as a result of chemical or biological reactivity. Although bleomycin is toxic[10,11], it was used here (below the toxicity level) because all the appropriate parameters such as toxicity, binding constants, and Doppler shift for the Mössbauer effect are known. The ideal drug would have no toxicity at all; it would simply bind the Mössbauer isotope to the DNA. The drug concentration that achieves a level of intercalation sufficient for this treatment can be three orders of magnitude lower than the LD_{50} in mice[12–17]. Furthermore, a conventional chemotherapeutic agent relies on a specific mechanism that constrains the effective structure of the agent and makes it subject to acquired tumour

Table 2 Effect of drug $^{57}Fe(III)$·bleomycin (no radiation) on C3H mice bearing spontaneous mammary adenocarcinomas

Mouse number	Initial tumour length (mm)	Initial tumour width (mm)	Final tumour length (mm)	Final tumour width (mm)	Initial tumour volume* (mm^3)	Final tumour volume* (mm^3)	Final vol/ initial vol	Initial weight (g)	Weight change (g)
15	10.0	8.0	18.3	14.6	320	1,950	6.09	29.4	1.4
16	8.0	5.4	22.3	17.5	117	3,415	29.2	29.1	2.9
17	5.0	5.0	14.5	11.1	62	893	14.3	27.0	−0.5
18	8.3	8.0	22.0	20.7	266	4,713	17.7	32.0	0.6
19	7.0	6.1	23.4	22.5	130	5,936	45.7	31.1	2.6
20	9.4	8.7	17.1	15.4	356	2,028	5.70	28.8	0.5
21	10.2	10.1	17.3	15.1	520	1,972	3.79	29.4	4.6
22	10.4	7.0	20.0	17.1	255	2,924	11.5	29.7	4.4
23	9.5	7.0	17.4	12.4	236	1,349	5.72	29.6	0
24	9.8	6.7	19.8	17.5	220	3,024	13.7	25.5	4.5
25	7.8	4.9	17.0	16.7	94	2,371	25.2	27.5	0.9
26	6.2	5.4	9.2	6.4	90	190	2.12	31.5	−0.3
27	7.7	5.5	13.3	8.5	116	480	4.14	32.5	0.3
						Average 14.4 ± 12 (s.d.)			1.7

All mice were anaesthetized with an intramuscular injection of 40 mg kg^{-1} ketamine, 5 mg kg^{-1} xylazine, and 0.03 mg kg^{-1} atropine. Four mg kg^{-1} drug $^{57}Fe(III)$·bleomycin was injected intratumorally (about 0.1 ml of 1.5 mg drug per ml). The mice were injected on days 1, 5, 9 and 13 and killed on day 17.

*Tumour volume was calculated as length × $width^2/2$.

Table 3 Effect of $^{57}Fe(III)$·bleomycin with Mössbauer radiation on C3H mice bearing spontaneous mammary adenocarcinomas

Mouse number	Initial tumour length (mm)	Initial tumour width (mm)	Final tumour length (mm)	Final tumour width (mm)	Initial tumour volume* (mm^3)	Final tumour volume* (mm^3)	Final vol/ initial vol	Initial weight (g)	Weight change (g)
1	10.5	10.5	9.1	8.0	579	291	0.503	29.0	−1.1
2	10.1	10.1	1.0	1.0	515	0.5	0.001	25.5	1.4
3	8.0	8.0	4.5	4.3	256	41	0.163	29.5	1.0
4	8.1	8.0	6.0	5.9	259	104	0.402	26.5	2.7
5	10.0	5.6	7.2	7.2	157	187	1.19	30.1	−0.8
6†	6.8	6.8	16.1	13.2	157	1,403	8.9	28.5	1.57
7	5.0	5.0	6.0	5.7	62	97	1.56	30.1	−3.5
8	13.0	11.2	11.8	9.9	811	578	0.713	31.5	−1.5
9	11.4	7.3	15.0	11.9	304	1,062	3.49	28.3	−0.5
10	10.6	7.2	11.8	10.0	277	593	2.14	30.8	−0.9
11	8.5	7.3	6.9	6.1	226	128	0.566	28.1	0.7
12	9.1	7.2	11.8	10.6	236	663	2.81	29.9	0.1
13	8.0	7.6	9.6	8.8	243	374	1.54	28.3	0.9
14	6.3	5.1	8.6	6.7	82	193	2.35	26.5	−1.0
							Average 1.9 ± 2.2 (s.d.)		−0.07

All mice were anaesthetized with an intramuscular injection of 40 mg kg^{-1} ketamine, 5 mg kg^{-1} xylazine, and 0.03 mg kg^{-1} atropine. Four mg kg^{-1} drug $^{57}Fe(III)$·bleomycin was injected intratumourally (about 0.1 ml of 1.5 mg drug per ml). The tumour was placed directly under the source and irradiated for 15 min. Mice were treated on days 1, 5, 9 and 13. The radiation dose was $\sim 1 \times 10^{-4}$ Gy per treatment (total) and $\sim 1 \times 10^{-5}$ Gy per treatment for the 14.4 keV gamma ray. The mice were killed on day 17.

* Tumour volume was calculated as in Table 2.

† Tumour was difficult to immobilize because of its location on the chest wall.

resistance. In this therapy, tumour resistance can be ameliorated because any Mössbauer-isotope molecule that binds tightly to the DNA should be effective.

Negligible radiation doses and pharmacological nontoxicity together have important implications in human therapy for the elimination of pathological cell populations. Furthermore, the ability to control the occurrence of the Mössbauer effect over small spatial dimensions by manipulation of the resonance conditions, with the application of a magnetic field for example, could be a basis for selective cell-eradication therapy in humans.

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