Inactivation of HIV by application of heat and radiation

Implication in bone banking with irradiated allograft bone

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ABSTRACT – We developed methods for inactivating the human immunodeficiency virus by heat and ionizing radiation and tested the effects of these treatments on the mechanical strength of bone. Simultaneous use of heat and radiation caused a considerably greater inactivation of HIV than the additive effects of the two separate treatments, but also caused a significant reduction in the maximum load sustained by the bone specimens tested with an Instrom machine. Application of the same doses but given in the sequential fashion of radiation followed by heat also caused marked inactivation of HIV and had less effect on the mechanical strength of the bone.

Among the different methods of bone sterilization, irradiation is one of the most popular (Turner et al. 1956, de Vries et al. 1958, Wright and Trump 1970, Hernigou et al. 1993) and several authors have reported the radiosensibility of human immunodeficiency virus (Spire et al. 1985, Kitchen et al. 1989, Conway et al. 1990, Campbell et al. 1994). In all studies, the dose required for inactivation of the virus has been calculated in frozen allografts. The irradiated specimens were kept at –70 °C. Treatments with heat or ionizing radiation are common methods of physical disinfection for inactivating viruses. However, certain viruses are very resistant to heat whereas others are extremely resistant to inactivation by ionizing radiation. In the case of HIV, heat inactivation is known to be effective (Spire et al. 1985). However, a study of intraosseous temperature during autoclaving (Böhm and Stihler 1995) has shown that disinfection or even sterilization of massive allografts cannot be guaranteed after 15 minutes at 134 °C (program of usual autoclaves). At this temperature, there is a significant decrease in the mechanical properties of human bone. The standard dose of radiation used to sterilize bone in tissue banks is 25 kGy (± 5 kGy), which is sufficient to reduce the surviving fraction of most bacteria to 10^{-9}, but not adequate for HIV. To obtain the same surviving fraction, the equivalent dose for HIV is reported to be 60 kGy or more. At this dose, there is also a significant decrease in the mechanical properties of human bone (Currey et al. 1997).

To obviate the need for high doses of heat or radiation to inactivate HIV in bone, a combination of both methods might be of value. HIV could be inactivated by both the heat and radiation components, since temperature affects the dose of radiation to inactivate viruses (Ward 1980). We investigated whether thiscombination could improve the inactivation of HIV in bone allografts, with an acceptable reduction in the mechanical integrity of the bone.

Material and methods

The virus

We employed the HIV-1/LAI as reference strain; it has always been cultured on normal human cells (and has therefore never been in contact with cell lines). To test viral virulence, a very sensitive cell

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line was used: MT2 cell line, a T lymphoblastoid cell line which is HTLV-I positive and is sensitive to HIV-1/LAI infection. It was maintained in RPMI 1640 culture medium containing 10% heat-inactivated fetal calf serum (Boehringer Mannheim), 2mM L-glutamine (Boehringer Mannheim) and 1% antibiotics (PSN 100 x, Gibco). Cell concentration was 100,000 cells per mL.

Irradiation was performed using an accelerator delivering electrons of 6.2 MeV providing several tens of kGy in a few seconds. Human tibiae were obtained from the bone bank. The intramedullary content was removed. A vial of virus stock was placed in each bone with an alanine dosimeter. The bone end was sealed with a lead plug to ensure that the least radioopaque window to the virus was one cortical width of bone. The dose delivered was checked by placing alanine dosimeters in the bone at the site of the HIV aliquots. Alanine is a solid amino acid which, when irradiated, gives rise to free radicals; these were counted using electron paramagnetic resonance (Olsen et al. 1990) (relative standard deviation of less than 1%). Experimental temperatures between –80 °C and +40 °C were tested (every 20 °C). We tested experimental doses of irradiation at 50, 40, 30 and 25 kGy. Effects of simultaneous and sequential applications of heat and radiation were evaluated.

Virus titration

Irradiated virus or mock-irradiated virus were sequentially threefold diluted in a final volume of 100 µL of culture medium. The inoculi were put in a microtiter plate. We assessed each virus dilution in 6 replicate wells. 30,000 MT2 cells in 100 µL of culture medium were then added to each well, and the plates were put in a humidified, 5% CO_2 incubator at 37 °C. Half of the culture medium was renewed twice weekly. We assessed viral replication in each dish by syncytium detection using an inverted phase contrast microscope. The culture was stopped when we could detect no new positive well over a 1-week period. Infectious titers of the differently-treated viruses were then calculated using Karber's formula and results were expressed as 50% tissue culture infective doses per mL of stock virus (TCID 50/mL). The sensitivity limit of our technique is 100 TCID 50/mL (see definitions in Appendix).

Table 1. Log reduction obtained in the experiments

	kGy			
Temp. °C	25	30	40	50
40	5.7	7.3	9.8	13
20	5.6	6.6	8.7	11
0	5.0	6.1	7.9	10
–20	4.7	5.3	7.3	7.9
–40	3.9	5.3	6.1	7.5
–60	3.4	4.5	5.9	6.9
–80	3.0	4.2	5.3	6.2

Bone strength measurement

Standard bone ring samples from the midshaft of the femur of 2 donors were prepared using a previously described technique (Hamer et al. 1996). The mechanical tests were done with a three-point bending jig in an Instrom mechanical testing machine (type 1026). The specimens were loaded to failure and the maximum load sustained or Wmax (N) was measured.

Results

Radiosensitivity of the virus

Effects of simultaneous application of heat and radiation when the viral cultures were placed in the tibiae (Table 1). In these experiments, the viral cultures were placed in the tibiae. Only the Log reduction obtained in the experiments is presented, in accord with the temperature of irradiation. For example, 25 kGy irradiation of 316,227 TCID 50/mL at –80 °C led to a titer reduction of 3 logs, with 316 TCID 50/mL remaining. According to the technique's sensitivity (100 TCID 50/mL) and the sensitivity with which the dose of irradiation can be measured (less than 1%), the D10 (see Appendix) value with "99% confidence limits" was 8.3 kGy. We observed that the degree of inactivation of HIV by irradiation was temperature dependent. For example, the log reduction in the number of TCID50 was 3.9 at –40 °C, with an irradiation of 25 kGy, and 5.56 at 20 °C, with an irradiation of 25 kGy.

Effects of sequential application of heat and radiation (Table 2). We measured the effects of heat and radiation after simultaneous and sequential

Table 2. Effects of heat and radiation on the log reduction of HIV

Treatment	Log reduction
50°C during 30 min	1.12
30 kGy at –80 °C	4.2
50°C, 30 min, followed by 30 kGy at –80 °C	5.19
30 kGy at –80 °C, followed by 50°C, 30 min	7.16
50 kGy for 30 min and 30 kGy given simultaneously	8.43
50 kGy at –80 °C	7.02

treatments in a second experiment. This was done to detect whether the simultaneous application of heat and radiation had a greater effect on inactivation than the additive effects of the 2 applications. Several temperatures (20 °C, 30 °C, 40 °C, 50 °C, 60 °C) were tried out. The most interesting temperature was found to be 50 °C. In this second experiment, the viral cultures were not placed in the bone specimens. Samples, except those given radiation alone, were heated at 50 °C for 30 min. Simultaneous application of heat and radiation resulted in an inactivation rate that was significantly greater (log reduction = 8.4) than the additive effects of the two separate applications. A similar effect (but less consistent) was also observed when applications were given in a radiation followed by heat sequence (log reduction = 7.2). When the applications were given in the opposite sequence, no effect was detectable (log reduction 5.2).

From the results of these experiments, we conclude that the doses of radiation required to inactivate HIV are considerably less when radiation and heat are given together either simultaneously or sequentially in a radiation followed by heat sequence, than would be required if only radiation was used at –80 °C (50 kGy for 7.02 log reduction).

Since heat and radiation given together may be the optimal method for inactivation of HIV, we also studied the effects on mechanical properties of the bone.

Effects of radiation and heat on the mechanical properties of human bone (Table 3)

Since the mechanical properties of bone might vary along its length, we examined pairs of specimens (one control, one with treatment) that were taken, as near as possible, from homologous positions in the two paired femurs. The data are representative of experiments that in each case were repeated 6 times. When heat and radiation were combined and given simultaneously, there was a greater reduction in the maximum load sustained than when they were given sequentially. From these results, we conclude that heat and radiation affect the maximum load less when given in sequence. Since inactivation was better when radiation was given after heat, this appears to be the best combination for inactivation of HIV and preservation of biomechanical properties in the allograft. We also did comparative studies of the maximum load sustained with doses of radiation (50 kGy) that caused at least as much inactivation of HIV as that produced with the combined heat and radiation treatment. A radiation dose alone of 50 kGy at –80 °C caused a much greater reduction in the maximum load than heat and radiation combined with the radiation after heat sequence.

Table 3. Bone strength measurements

Treatment	W max (N) [a] mean	Treated/control mean percentage (SD)	No. of experiments
Control	51		
50°C during 30 min	49	96 (8)	6
30 kGy at –80 °C	46	87 (7)	6
50 °C, 30 min, followed by 30 kGy at –80 °C	43	84 (9)	6
30 kGy at –80 °C, followed by 50 °C, 30 min	47	90 (10)	6
50 kGy for 30 min and 30 kGy given simultaneously	29	61 (6)	6
50 kGy at –80 °CC	17	33 (5)	6

[a] load at failure

Discussion

Since 1985, when HIV screening of blood started, 4 cases of transmission of HIV through bone transplantation have been reported in USA. The first case was reported in 1988 (Center of Disease Control 1988). The other 3 cases, reported in 1992 (Simonds et al. 1992), were related to a single donor. This shows that the limitations of donor screening result in a finite but real risk of viral transmission through bone allografts and that methods to reduce the risk must be assessed.

Among the different methods of sterilization, irradiation is one of the most popular and several authors have reported the radiosensitivity of the virus. However, they have recommended doses rangingfrom 10 kGy to 40 kGy to destroy the virus. Furthermore, patients with infection may have different amounts of virus in bone depending on their plasma viral load and on the type of bone (cortical, marrow). Thus, a dose of irradiation that may sterilize the bone from one donor may not be the dose required for another bone from the same donor or for a bone from another donor. It is therefore difficult to determine the efficacy of irradiation in eliminating the risk of transmission of HIV by allografts and all the methods to reduce the risk must be carefullly evaluated.

Fideler et al. (1994) studied the effects of various doses of radiation, ranging from 20 to 40 kGy, with respect to the inactivation of HIV in frozen allografts at –70 °C. He concluded that a dose of 30 kGy was necessary for sterilization of the graft. In our study, 30 kGy at –70 °C gives a 3 log reduction. However, 30 kGy at 0 °C gives 6 log reductions but 25 kGy at 40 °C also gives a 6 log reductions. Since simultaneous application of heat and radiation causes considerable inactivation of HIV, bone allografts should be irradiated at the ambient temperature rather than at –70 °C. The cumulative effects of temperature and irradiation on viruses indicate that a lower dose can be used if irradiation is given at the ambient temperature.

If irradiation was completely harmless to tissue, the solution would be simply to irradiate the tissue with doses that kill the highest potential bioburden. However, high dose radiation can be harmful for the tissue: it reduces ossification and compromises strength. The maximum level of irradiation will therefore depend on the mechanical tests and the degree of loss of mechanical properties that is acceptable. For bone, the mechanical properties are affected between 25 kGy and 60 kGy, i.e., in the dose range for sterilization. The mechanical properties are also affected by the temperature of irradiation (Hamer et al. 1999). To avoid undue exposure of grafts to irradiation and make the irradiation process efficient, it is essential to choose the correct radiation dose and temperature.

Although simultaneous application of heat and radiation causes marked inactivation of HIV, it can also cause more damage to the mechanical properties of the bone than the sequential treatment. Sequential sterilization of bone given in the radiation-followed-by-heat sequence caused a better inactivation than the same application given in reverse order; the reduction in the mechanical properties was acceptable as compared to the reduction obtained when only irradiation was given to obtain the same inactivation of HIV. The causes of this effect of the radiation-followed-by-heat sequence are unclear. The mechanical properties are probably linked to collagen denaturation. HIV and collagen are both damaged by radiation and heat. However, these two kinds of proteins probably do not have the same resistance to heat and radiation. This may explain the differences in effects observed according to the sequences of heat and radiation.

On the basis of our findings, we recommend 30 kGy radiation followed by 50 °C for 30 min. In clinical practice, the allografts are subjected to varying loading rates according to their situation. Furthermore, during the biological phenomenon of incorporation, the strength may vary depending on the mineral fraction, the collagen fraction and on the interaction of both. Even if irradiation is given in a dose which does not reduce the mechanical strength too much, failure may occur later.

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Appendix

Definitions

D10: The D 10 dose is the decimal reduction dose equal to the number of grays necessary to reduce the surviving viral population to 1/10 of the initial population. Since the log decimal (1/10) is equal to –1, D10 is also called the dose of radiation required to reduce the population by one log cycle.

TCID 50: Quantity of virus giving infection in 50% of cultures. 1 virus is different from 1 TCID 50 and there is no exact equivalence between these two units. 1 virus may be considered to be equivalent to 1.44 TCID 50 (KARBER law), but this is not exact. Infectious titers are calculated with KARBER's formula and are expressed as 50 % tissue culture infective dose per milliliter of stock virus (TCID 50/ml) or simply TCID 50.

1 Megarad: 10 kGy.