Restoring Natural Killer Cell Cytotoxicity After Hyperthermia Alone or Combined with Radiotherapy

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Abstract. *Background: The aim of the present study was to investigate in vitro the effect of hypo- and hyperthermia alone or in combination with irradiation on natural killer cell (NK) cytotoxicity, recovery of this function and the possibility of preventing damage to or enhancing cytotoxicity recovery using interferons (IFNs) α, β, and γ and interleukin-2 (IL-2). Materials and Methods: We used non-selected NK cells and measured their cytotoxicity using the ^{51}Cr release assay. Temperatures ranging from 31-45˚C and thermal treatment times from 0-180 min were assessed. IFNs were applied at concentrations from 0-1,000 IU/ml and IL-2 from 0-450 IU/ml. The range of irradiation dose was from 0-30 Gy. Results: We detected no significant differences in cytotoxicity at temperatures from 31-37˚C. The most significant decrease in cytotoxicity was observed between 41 and 42˚C (p=0.0010), and heating NK cells at 42˚C for 180 min almost completely abolished this function. NK cell cytotoxicity largely recovered during the first 24 h, depending on the heating time. IFN-α, β, and γ demonstrated no concentration-dependent ability to aid in recovery when used before or after the thermal treatment. In contrast, IL-2 restored cytotoxicity in a concentration- and incubation time-dependent manner and was equally active when used before, during or after heating. NK cells were heated at 42˚C for various times and then irradiated with a single dose or first irradiated and then heated; however, no statistically significant differences were observed (p=0.520). An approach of IL-2 treatment followed by radiation and heating was the most effective in restoring NK cytotoxicity (p=0.000). Conclusion: NK cell cytotoxicity is impaired in vitro at 42˚C and above, with possible partial recovery. IL-2, but not IFNs, was able to restore NK cell cytotoxicity in a concentration-dependent manner. IL-2 can also reverse the damage caused by combined hyperthermia and irradiation.*

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In humans, hyperthermia is defined as a temperature greater than 37.5 or 38.3˚C (99.5-100.9˚F), depending on the publication (1, 2). Hyperthermia causes various changes in cellular macromolecules, including unfolding and aggregation of proteins as well as DNA damage (3). It is well-known that hyperthermia kills both normal (4) and tumor cells (5, 6), though certain types of cancer are more sensitive to hyperthermia than normal cells (7). In addition, hyperthermia has an influence on blood flow and on the tumor microenvironment *in vivo* and can act as an adjuvant for the immune system (8).

Indeed, hyperthermia was one of the first therapies used to treat cancer (9), and hyperthermia is currently applied as a systemic treatment to the entire body, local and regional or as ablation treatment (tissue burning) (10). Several approaches can be utilized to induce hyperthermia, including conduction, convection and radiation techniques. Also, bioactive, chemical, mechanical or electromagnetic techniques, with or without nanoparticles, have been used non-invasively, semi-invasively or invasively (10). For cancer therapy, hyperthermia can be applied alone (11) as well as combined with cytokines (12), radiotherapy (13, 14) or chemotherapy (15, 16). However, to date, there exist no reports regarding the *in vivo* combination of hyperthermia, radiotherapy, and cytokines with or without cytostatic drugs.

Temperatures from 38-42˚C are applied for whole-body hyperthermia, whereas 40-45˚C is typically used for loco-regional therapies. Conversely, higher temperatures, ranging from 60-250˚C, are utilized in ablative treatment. The heating times to reach the steady state are from 30 to 180 min in systemic therapy and the entire treatment time from 120 to 720 min. The corresponding times for local/regional treatments vary from 5 to 50 min and from 30 to 90 min, respectively (10).

Natural killer (NK) cells constitute one of the body's non-specific defenses against tumor cells and microbial pathogens (17), and depending on the heating time, *in vivo* NK cell activity and blood levels can be enhanced at temperatures within the fever range (38.3-42˚C) (21-23). However, *in vitro* NK cell viability and cytotoxicity are decreased at higher temperatures (18, 19). NK cell-induced tumor lysis itself is more thermosensitive than the recognition and binding functions of NK cells (20). Further investigations into the roles of major histocompatibility complex (MHC) class I, MHC Class I-like molecule (MICA), heat shock proteins (HPS), NK cell surface natural-killer group 2, member D (NKG2D) and lipid raft clustering (21) have been reported.

Whole-body hyperthermia increases IL-2 and NK cell activity (22, 23). Hypothermia also enhances NK cell activity in healthy individuals at temperatures as low as 35˚C (24, 25), though activity of these cells was reportedly decreased during perioperative hypothermia (26).

In the present study, we systematically examined the effects of hypothermia and hyperthermia (31-45˚C) and heating times on the cytotoxicity of non-selected NK cells *in vitro*, as measured using a ^{51}Cr release assay. In addition, we assessed the effects of IFN-α, β and γ as well as IL-2 in preventing thermal damage to NK cells and their recovery. The combined effects of thermal treatment and radiation on NK cell cytotoxicity and the ability of IL-2 to reverse the damage incurred were also investigated.

Materials and Methods

NK cell enrichment. NK cells were enriched from buffy coat samples obtained from the Finnish Red Cross Transfusion laboratory, with permission of the ethical committee of the Finnish Red Cross Blood Service (customer number 6129, approval number 331/2013, tutkijaluvat@veripalvelu.fi) in accordance with the Finnish law.

We used a method previously described to isolate non-selected NK cells (method II in Hietanen *et al*. 2015) (27). Briefly, peripheral blood mononuclear cells (PBMCs) were isolated by a Ficoll-Paque gradient, plastic and nylon wool adherence and a discontinuous Percoll gradient. The yield, purity, viability, cytotoxicity and radiation sensitivity of the enriched NK cells was described in a previous report (27).

Thermal treatment. NK and target K-562 cells were suspended in 1 ml of medium and incubated at different temperatures in a water bath or an incubator in a 5% CO_2 and humified atmosphere for time periods typically ranging from 0-180 min. Preliminary studies showed no differences between the results obtained using a water bath or an incubator. Temperatures were measured using a Fluke 51 K/J thermometer (John Fluke, Everet, WA, USA) with an accuracy of +/–0.1˚C, and pH was controlled with phenyl red present in the medium. Cytotoxicity measurements were performed immediately after the thermal treatment, with the exception of recovery studies, for which they were performed up to 72 h after the thermal treatment.

Irradiation. The enriched NK cell populations were gamma-irradiated, as described earlier (28). In brief, we used a ^{137}Cs device (GAMMACELL 2000, Mølsgaard, Denmark) at a dose rate of 4.1 Gy/min at room temperature (20˚C). Well-oxygenated NK cells were irradiated in U-bottom plastic tubes (BD Falcon, Franklin Lakes, NJ, USA), and the dose was controlled using lithium fluoride thermoluminescence dosimetry. NK cells were irradiated using single doses from 0 to 30 Gy.

IFN α, β γ and IL-2. We used recombinant IFN-α2b (Schering-Plough, Kenilworth, NJ, USA), recombinant IFN-β (Kyova Hakko Kogyo Co, Ltd, Tokyo, Japan), recombinant IFN-γ (Genenteck, San Francisco, CA, USA), and recombinant IL-2 (Janssen Biochimica, Beerse, Belgium), as reported previously (28). In brief, we incubated NK cells with IFN-α, β and γ at concentrations ranging from 1 to 1,000 U/ml for 24 h at 37˚C before or after heat treatment. NK cells were incubated with IL-2 for times ranging from 0-140 h at 1.75 to 450 IU/ml before or after heating at 37˚C and during heating at the indicated temperatures. Samples of 1.25×10^6 NK cells/0.9 ml medium were incubated with 0.1 ml IFNs or various concentrations of IL-2 in U-bottomed, 10 ml plastic tubes. Controls were treated in the same manner, with 0.1 ml of 0.9% NaCl instead of cytokines.

Measurement of NK cell killing capacity. The cytotoxicity of NK cells was measured using a modified ^{51}Cr release assay (29). Briefly, we used a target:effector ratio of 1:12.5. The effector NK cells were thermal treated with or without cytokines and/or irradiation, and measurements were performed in triplicate. Cells in 96-well U-bottomed microtiter plates were incubated for 18 hours at 37˚C in an incubator with a humified atmosphere containing 5% CO_2. The harvested supernatants were measured using a gamma counter (1272 Clinigamma, LKB Wallac, Turku, Finland). The relative cytotoxicity was calculated using the following formula: Cx (%)=((exp.-spont.)/(max.-spont.) ×100), where exp. is the average of the experimental wells, spont. is the spontaneous release, and max. is the maximal release from K-562 cells lysed with 1% Triton X-100. The mean values of triplicate samples were used in further calculations. The results were expressed as a percentage of non-heated, non-irradiated and non-cytokine-treated controls.

Cells of an erythroleukemia cell line K-562 (30) were used as the target cells. The cells were grown in RPMI 1640 (Orion Diagnostica, Espoo, Finland) with 10% temperature-inactivated fetal calf serum (Flow Laboratories, Irvine, Scotland), 0.3 g/l L(+) glutamine (Fluka, Buchs, Switzerland) and 20 mg/ml gentamicin (Flow Laboratories, Irvine, Scotland).

Data presentation and statistical analyses. The results of the ^{51}Cr release assays were expressed as a percentage of the unheated NK cell controls at 37˚C. Every value in this report represents the mean of several independent tests using material from different donors. The results are presented as the means±standard deviation (S.D.). The data were analyzed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). We used analysis of variance (ANOVA) to test the significance of differences between the effects of the different temperatures and thermal treatment times with or without cytokines and irradiation. *Post hoc* analysis was performed using Bonferroni correction of *t*-test results. The constituent ratios were compared using the χ^2 test (Chi-square test) and the Fisher's exact test. The Kruskal-Wallis test and the Mann-Whitney *U*-test were used for data

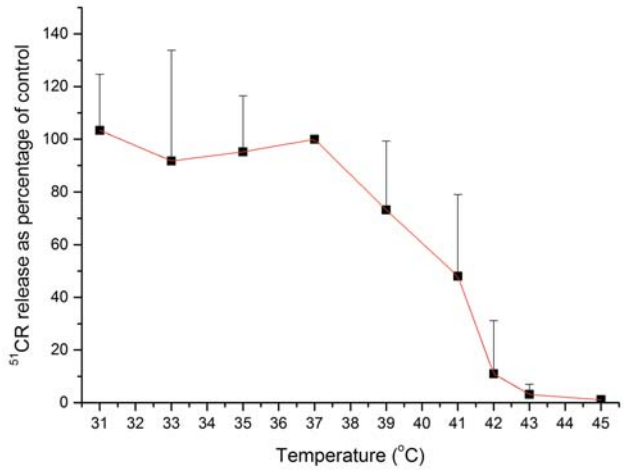

Figure 1. *Cytotoxicity of NK cells at different temperatures. NK cells were incubated for 180 min at temperatures ranging from 31 to 45˚C. The error bars represent the standard deviation of the experiments (n=2-27).*

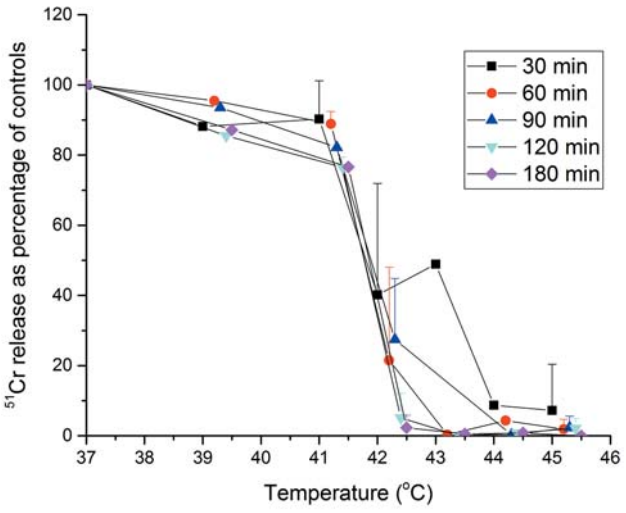

Figure 2. *The effects of different incubation times and temperatures on NK cell cytotoxicity. NK cells were incubated for 30, 60, 90, 120 and 180 min at temperatures ranging from 39 to 45˚C, and cytotoxicity was measured. The error bars represent the standard deviation of the experiments (n=2-27).*

that could not be assessed *via* ANOVA (*e.g.*, data that were not normally distributed). Differences were considered to be significant at $p<0.05$ (two-sided).

Results

Effect of temperature and heating time on NK cell cytotoxicity. The effect of temperatures ranging from 31 to 45˚C for 180 min on NK cell cytotoxicity was investigated in this study. Although there was no change in cytotoxicity from 31 to 37˚C, a continuous decrease in cytotoxicity was observed above this temperature, and cytotoxicity was near zero at 43˚C (Figure 1).

In ensuing experiments, temperatures ranging from 39 to 42˚C were studied in more detail. NK cells were incubated for 30, 60, 90, 120 and 180 min at these temperatures, and cytotoxicity was measured. Temperature elevation from 39˚C to 41˚C did not significantly affect ^{51}Cr release levels ($p=0.318$) at any heating time, whereas an increase from 41˚C to 42˚C resulted in a clear reduction of ^{51}Cr release ($p=0.010$) at all heating times (Figure 2).

To explore the duration of hyperthermic treatment necessary for an effect on the cytotoxicity of non-selected NK cells, cells were incubated at 42˚C for various times, ranging from 0 to 180 min. We found that cytotoxicity decreased almost linearly at a logarithmic scale, reaching almost 0% of the cytotoxicity of control cells after 180 min (Figure 3).

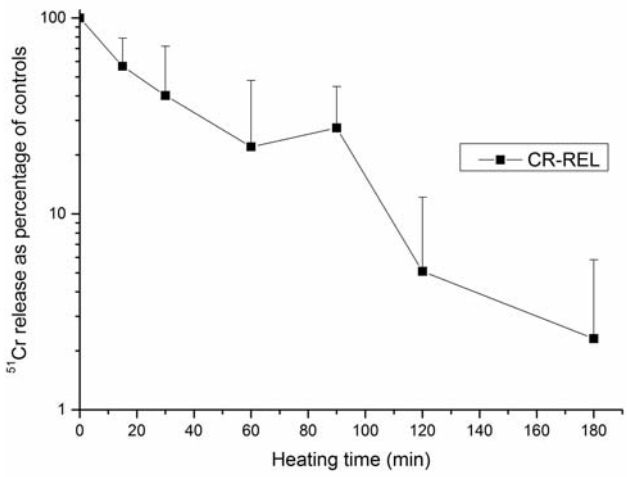

Figure 3. *Effect of different thermal treatment times at 42˚C on the cytotoxicity of NK cells. NK cells were heated at 42˚C for 0, 15, 30, 60, 90, 120 and 180 min, and cytotoxicity was evaluated. The error bars represent the standard deviation of the experiments (n=12-24).*

Recovery of NK cell cytotoxicity after thermal treatment. The recovery of NK cell cytotoxicity after thermal injury was examined by heating NK cells at 42˚C for 0, 30, 60, 120 and 180 min; the cells were then incubated for 24, 48 and 72 h at 37˚C. For all thermal treatment times, the major portion of recovery occurred within the first 24 h. An increase in cytotoxicity was significant at all recovery times ($p=0.011$, 0.014 and 0.011, respectively), with a slow increase up to 48 h observed, except for the longest heating time (180 min) (Figure 4A). For a heating time of 30 min, 70% of the cytotoxicity was restored at 72 h.

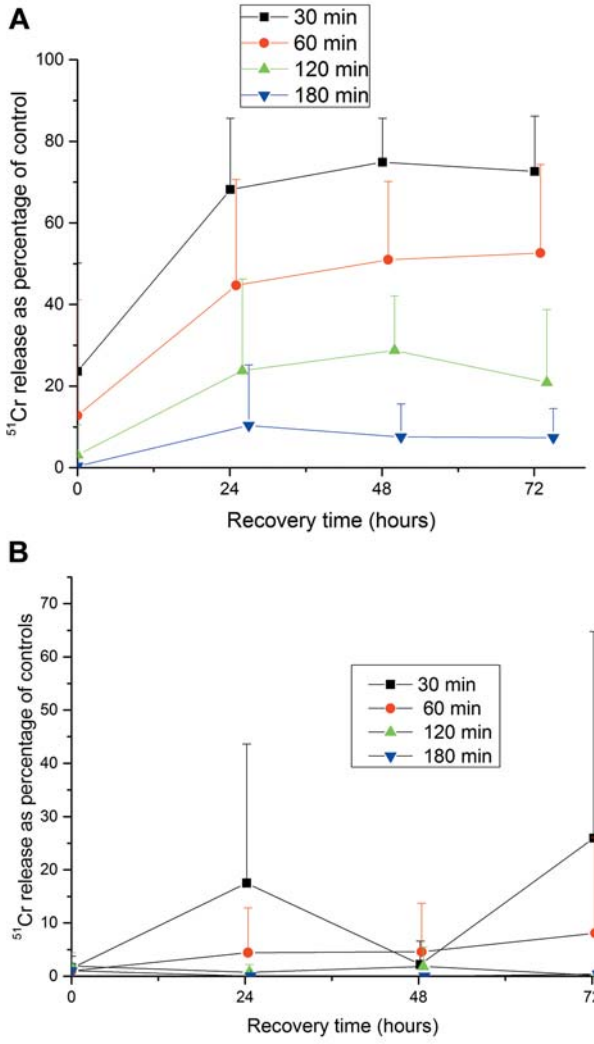

Figure 4. *Recovery of NK cell cytotoxicity after thermal treatment for different times at 42˚C (A) and 45˚C (B). NK cells were incubated at 42˚C (A) and 45˚C (B) for 0, 30, 60, 120 and 180 min. Recovery times were 24, 48 and 72 h at 37˚C. Cytotoxicity was measured. The error bars represent the standard deviation of the experiments (n=4).*

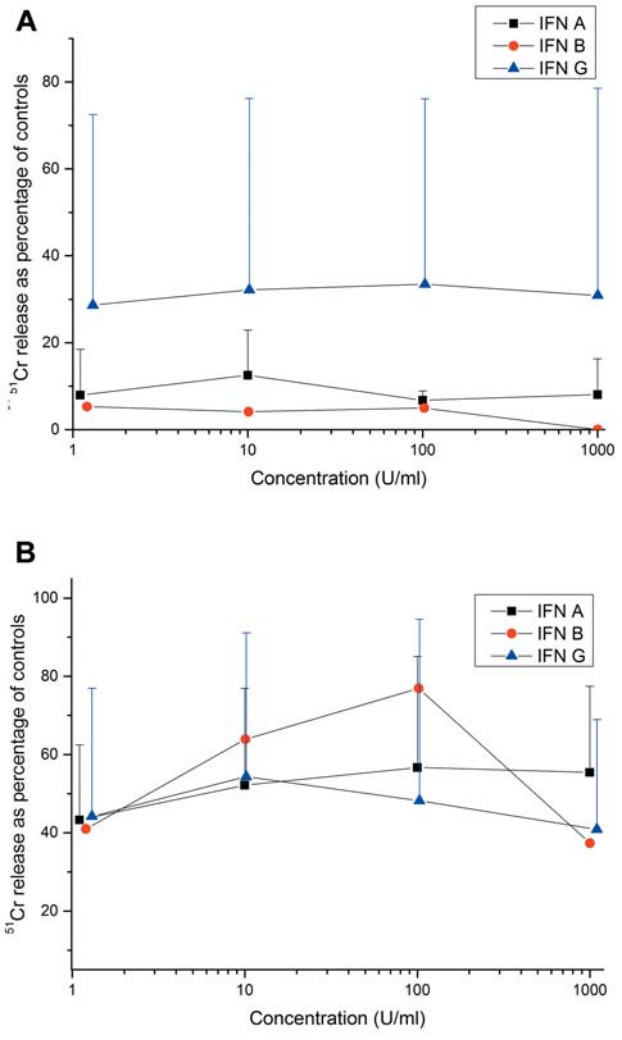

Figure 5. *The role of IFN-α, β, and γ in preventing thermal damage (A) and restoring cytotoxicity (B) to NK cells. The ability of IFN-α,β and γ to prevent thermal damage to NK cells or restore cytotoxicity is presented. Cells were first incubated at 37˚C for 24 hours with IFN α,β and γ at concentrations from 1 to 1000 UI/ml and then treated for 180 min at 42˚C (A). Next, incubation with interferons was conducted after thermal treatment (B). Cytotoxicities were measured. The error bars represent the standard deviation of the experiments (n=2-4).*

The same thermal treatment was conducted at 45˚C, and the restoration of cytotoxicity was much lower than at 42˚C. Indeed, the best recovery of cytotoxicity after 72 h was at most only 25% of the controls after 30 min of thermal treatment (Figure 4B).

The role of IFN α, β, γ and IL-2 in preventing thermal injury and restoring the cytotoxicity of NK cells. To study the role of IFN α, β, and γ in preventing thermal injury, NK cells were incubated for 24 h at 37˚C with 1 to 1000 UI/ml IFN α, β, γ. Thereafter, the cells were heated at 42˚C for 180 min. In these experiments, IFN α, β, and γ exhibited no concentration-dependent effect on cytotoxicity when applied before the thermal treatment (Figure 5A).

Next, the effects of IFN-α, β and γ treatment on NK cell recovery after thermal damage were evaluated, with incubation for 24 h after heating at 42˚C. Again, interferons showed no statistically significant concentration-dependent effects of the recovery of NK cell cytotoxicity (Figure 5B). Although the interferons showed no significant effect on the restoration of NK cell cytotoxicity, recovery was slightly more pronounced when these cytokines were applied after heating.

To explore the ability of IL-2 to prevent thermal damage, the IL-2 concentrations and incubation times necessary for an

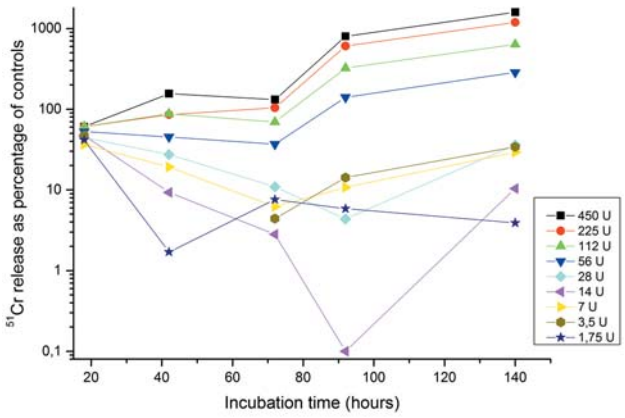

Figure 6. *NK cell cytotoxicity as a function of IL-2 concentration and incubation time at 42˚C before hyperthermia treatment. NK cells were incubated for 0, 18, 42, 72, 92 and 140 hours at 37˚C with IL-2 at concentrations from 0 to 450 U/ml. Subsequently, the cells were subjected to thermal treatment at 4˚C for 3 h. The recovery of cytotoxicity is presented (n=1).*

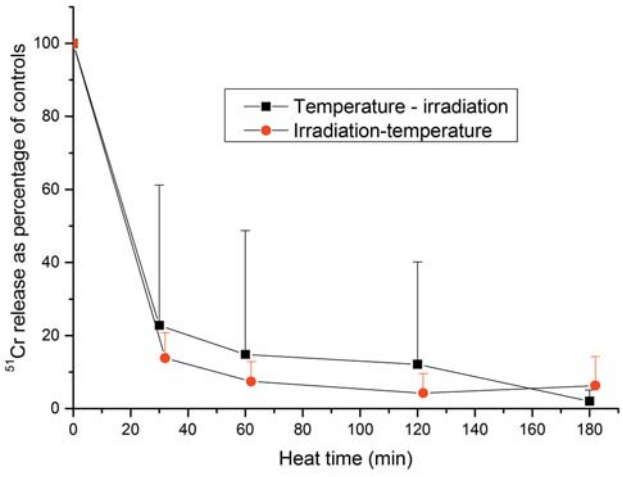

Figure 8. *The effect of the order of different heating times at 42˚C and irradiation on NK cell cytotoxicity. NK cells were treated at 42˚C for different heating times and irradiated with 20 Gy. The following sequences were used: thermal treatment first, then irradiation (temperature-irradiation) and vice versa (irradiation-temperature). The error bars represent the standard deviation of the experiments (n≥6).*

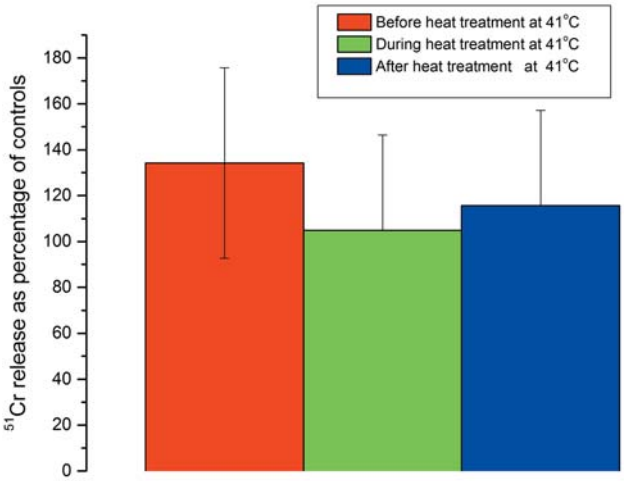

Figure 7. *NK cell cytotoxicity with IL-2 treatment before, during and after thermal treatment at 41˚C. NK cells were incubated with IL-2 at 450 UI/ml for 18 h before, during or after 3 h thermal treatment at a temperature of 41˚C. The error bars represent the standard deviation of the experiments (n=4-5).*

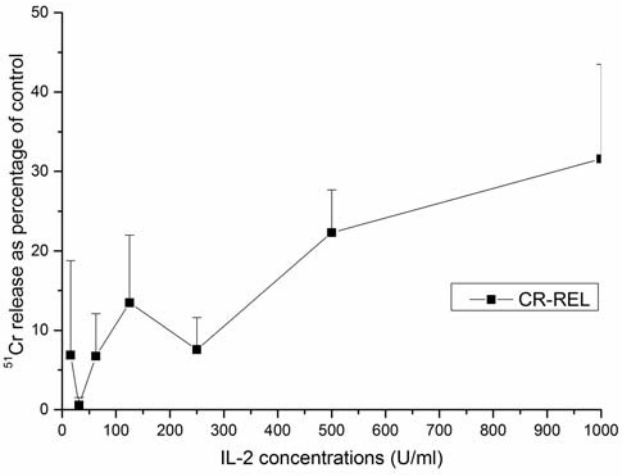

Figure 9. *Effect of different concentrations of IL-2 on NK cell cytotoxicity irradiated with 20 Gy and heated at 42˚C. NK cells were first irradiated with 20 Gy, then heated at 42˚C for 60 min and finally incubated with IL-2 at concentrations from 0 to 1,000 UI/ml. The error bars represent the standard deviation of the experiments (n=3).*

effect were determined. NK cells were incubated from 0 to 140 h with 0 to 450 IU/ml IL-2 and then heated for 180 min at 42˚C. Concentrations of 28 IU/ml or less were not able to fully restore cytotoxicity, whereas concentrations ≥56 IU/ml markedly increased cytotoxicity over 100% of the control. This elevation was strongly dependent on both the IL-2 concentration and incubation time. The highest cytotoxicity value, 1585% of the control, was achieved by using 450 IU/ml IL-2 and an incubation time of 140 h (Figure 6).

Next, we assessed the time of IL-2 treatment in relation to heating by incubating NK cells at 37˚C for 18 h with 450 UI/ml IL-2 before, during or after thermal treatment at 41˚C for 180 min. However, no statistically significant differences between the timing of IL-2 treatment and the heating were observed (p=0.560) (Figure 7).

According to our results, interferons showed a very low and no concentration-dependent effect on NK cell cytotoxicity, whereas IL-2 was clearly effective in a

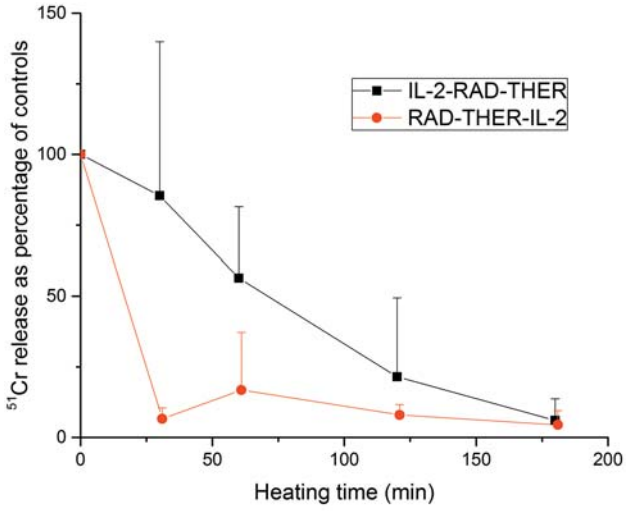

Figure 10. *The cytotoxicity of NK cells treated at 42˚C using combinations of IL-2 treatment, irradiation and hyperthermia. NK cells were incubated with 100 U/I IL-2 for 5 days at 37˚C. The irradiation dose was 20 Gy. Heating times at 42˚C were 0, 30, 60, 120 and 180 min. The following combinations were used: 1) IL-2, irradiation and hyperthermia (IL-2-RAD-THER) and 2) Irradiation, hyperthermia and IL-2 (RAD-THER-IL-2). The error bars represent the standard deviation of the experiments (n=3-12).*

concentration and incubation time-dependent manner. Therefore, IL-2 was the only cytokine selected for the next experiments.

Combined effect of hyperthermia and irradiation on NK cell cytotoxicity. First, NK cells were irradiated with doses from 0 to 30 Gy and then heated at 42˚C for 30 min. Based on its position in the middle of the cytotoxicity dose-response curve, the irradiation dose of 20 Gy was selected for further analysis. NK cells were irradiated at room temperature and then heated at 42˚C for 30, 60, 120 and 180 min. Two approaches were used, either irradiation first-followed by immediate thermal treatment or *vice versa*. No statistically significant differences in NK cell cytotoxicity were observed between the approaches at any heating time (p=0.520) (Figure 8).

In the following experiments, we used the same approaches with 0, 30, 60, 120 and 180 min intervals at 37˚C between the heating and irradiation treatments. Again, we found no statistically significant differences in cytotoxicity at any of the times examined (p=0.280, 0.140, 1.000 and 1.000, respectively).

IL-2 in the recovery of NK cell cytotoxicity from combined hyperthermia and irradiation. The combined effects of irradiation and cytokines on the cytotoxicity of NK cells are described in our earlier study (28). In the present study, we explored whether it was possible to reverse the effects of damage due to combined irradiation and thermal treatment on the cytotoxicity of NK cells.

Firstly, NK cells were incubated for 5 days with 100 UI/ml IL-2, a concentration based on the cytotoxicity dose-response curves of NK cells irradiated, heated and incubated with various IL-2 concentrations (Figure 9). The cells were then irradiated with 20 Gy and heated at 42˚C for 0 to 180 min. The irradiation dose was selected as described above.

Secondly, NK cells were incubated with IL-2 after irradiation and thermal treatment. A significant difference in cytotoxicity was observed when IL-2 was applied before irradiation and heating (p=0.000). Specifically, we found that the restoration of NK cell cytotoxicity was significantly improved with heating times of 30 and 60 min (p=0.030 and 0.000, respectively). However, with longer heating times, the IL-2 in concentration used was not able to significantly prevent the damage caused by irradiation and thermal treatment (Figure 10).

Discussion

We studied the *in vitro* thermal effects on non-selected NK cell cytotoxicity as a function of temperature and with a special focus on heating time and did not observe any significant differences in cytotoxicity within the temperature range from 31 to 37˚C. In our *in vitro* study, we detected neither the immunostimulating effects of acute cold exposure found *in vivo* (25) nor the decrease observed during perioperative hypothermia (26). The most significant decrease in cytotoxicity was observed between 41˚C and 42˚C, and this was strongly dependent on the heating time. In fact, cytotoxicity was almost completely destroyed after heating for 180 min at 42˚C, in agreement with previously published results (18, 31).

After heating times from 0 to 120 min at 42˚C, cytotoxicity was partially recovered, mostly during the first 24 h. However, there was almost no recovery at 45˚C, even with short heating times, as also reported in the literature (20).

In vitro, IFN-α, β or γ showed no dose-dependent effects in preventing or recovering NK cell cytotoxicity from thermal damage, though conflicting results have been published *in vivo*. Robins *et al.* (32) demonstrated that whole-body hyperthermia at 40.5˚C and IFN-γ therapy increases NK cell cytotoxicity, and Payne *et al.* (33) found that mild hyperthermia suppresses interferon-mediated enhancement of NK cell activity in human and murine cells. Conversely, Lamon *et al.* (34) reported the induction of thermotolerance in murine NK cells with IFN-α.

In our study, IL-2 exhibited a dose-dependent enhancement of NK cell cytotoxicity: at concentrations of 56 IU/ml and above, it was capable of fully restoring NK cell cytotoxicity after heating for 180 min at 42˚C. In addition,

there were no significant differences in NK cytotoxicity when IL-2 treatments were applied before, during or after heating. Kappel *et al*. (22) reported similar results at a slightly lower temperature of 39.5˚C.

When treating various cancers using a combination of whole-body heating and irradiation, the temperature usually ranges from 38˚C to 42˚C, and heating times from 40 to 60 min and daily irradiation doses from 1.8-2 Gy (even from 5-10 Gy for superficial tumors) have been applied (13). Thus, the temperature, irradiation and time range of our *in vitro* experiments are the same as those used *in vivo*. In some studies, thermal treatment was applied first, whereas the irradiation treatment was first in others (35). Our approaches included first heating at 42˚C followed by irradiation and vice versa, with no significant differences regarding NK cytotoxicity.

In addition, we investigated whether IL-2 is capable of preventing or restoring NK cell cytotoxicity after damage induced by combined hyperthermia and irradiation, and the recovery from thermal and irradiation damage was significantly higher when IL-2 was applied before heating and irradiation, results that were strongly dependent on heating time. Furthermore, various time intervals between irradiation and heating did not significantly affect the damage to cytotoxicity. We could not find any published study to date, *in vivo* or *in vitro*, on NK cell recovery with IL-2 after combined hyperthermia and irradiation.

The proportion of destroyed normal and tumor cells increases with increasing temperature (7). However, temperatures of 42.0-42.5˚C cannot be exceeded in whole-body cancer treatments. Temperatures up to 42˚C and treatment times from 40 to 60 min per session have been used in whole-body hyperthermia treatment of different cancer types, and an irradiation dose from 1.5 to 2 Gy per session can be added to the treatment. Under these conditions, our *in vitro* results revealed reduced NK cell cytotoxicity that was able to recover at least partially by itself and completely with IL-2. Depending on the individual's age and measurement method, IL-2 levels in the blood of healthy subjects range from 6 to 20 pg/ml (36), values that are less than 1 IU/ml. Theoretically, IL-2 could be applied in patients to reach levels high enough to protect NK cell cytotoxicity from the damage induced by hyperthermia and irradiation. Of course, there are other factors *in vivo* involved in NK cell recovery (8).

Since 1970´s the thermobiological rationale of hyperthermia has developed enormously. Hyperthermia is used clinically to enhance radiation and chemotherapy effects leading to thermoradiobiological and thermoradiochemotherapeutic rationales. Therapeutic whole-body as well as local hyperthermia induces both innate and adaptive anti-tumor immune responses including activation of the NK cells. Substantial roles in increasing the use of hyperthermia and better treatment results are the advanced hyperthermia treatment planning, execution and thermometry (37). In addition, during the last years the importance of immuno-oncology has been re-appraised, when targeted agents could be used to increase antigen dependent cellular cytotoxicity (38) and especially to restore immunological defense against cancer cells (39-42). In addition to IL-2 these new agents might be used during RT to increase cancer cell killing. However, clinical studies are warranted. Rosenberg and co-workers are still investigating better ways to clinically apply personalized immunotherapy for human cancers (43). In their studies the key cell populations are tumor-infiltrating lymphocytes (TILs) and NK cells and one of the key questions is how to increase cytotoxicity of these cells.

Conclusion

The effects of thermal and irradiation treatment on NK cell cytotoxicity were studied *in vitro*. No changes in cytotoxicity were detected at a temperature range from 31˚C to 37˚C, though elevation of the temperature from 41˚C to 42˚C significantly decreased cytotoxicity depending on the heating time. The recovery from the thermal injury by NK cells showed a temperature and heating time-dependent tendency. Although IFN-α, β and γ did not exhibit any concentration-dependent ability to prevent or recover heating damage, IL-2 was able to restore the damage partially or completely in a concentration-dependent manner when applied before, during or after thermal treatment. The impairment of NK cell cytotoxicity by the combination of hyperthermia and irradiation was dependent on the heating temperature and time as well as the irradiation dose, though there was no difference in the loss of cytotoxicity when the hyperthermic treatment was given before irradiation or vice versa. IL-2 was able to restore cytotoxicity more significantly when applied before the combined irradiation and thermal treatment.

Conflicts of Interest

The Authors declare no conflicts of interest.

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