0014-4754/96/050469-05$1.50 + 0.20

Accumulation of purine catabolites in solid tumors exposed to therapeutic hyperthermia

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Abstract. Intensified adenosine triphosphate (ATP) degradation following therapeutic hyperthermia is often observed in solid tumors. As a result, accumulation of purine catabolites can be expected together with formation of protons at several stages during degradation to the final product, uric acid. Proton formation in turn can contribute to the development of heat-induced acidosis. Furthermore, oxidation of hypoxanthine and xanthine may result in generation of reactive oxygen species, which may lead to DNA damage, lipid peroxidation and protein denaturation, thus also contributing to heat-induced cytotoxicity. In hyperthermia experiments a tumor-size-dependent, significant increase in the levels of the following catabolites has been demonstrated: Σ[IMP + GMP] (sum of guanosine and inosine monophosphate levels), inosine, hypoxanthine, xanthine and uric acid, along with a drop in ATP and guanosine triphosphate (GTP) levels. These data suggest that formation of reactive oxygen species and protons during purine degradation may indeed play a significant role in the antitumor effect of hyperthermia.
Key words. Tumor hyperthermia; ATP degradation; purine catabolism; hypoxanthine; xanthine; uric acid; reactive oxygen species; tumor acidosis.

Introduction

There are two major rationales for hyperthermia as an adjuvant modality in cancer therapy: differential heating and differential thermal sensitivity between malignant and normal tissues. These rationales are preferentially based on differences in blood flow rates, determining (1) heat dissipation from the heated tissue volume and (2) the metabolic microenvironment through limitations of the availability and clearance of metabolites[4, 19, 20–22]. In this context, cellular ATP depletion is thought to sensitize tumor cells to therapeutic hyperthermia[5, 7, 10]. A net decrease in ATP concentrations is often observed in experimental tumors exposed to therapeutically relevant levels or doses of hyperthermia[11, 16, 24]. This ATP depletion is mostly due to an intensified ATP hydrolysis (increased ATP turnover rate) during heating, a progressive restriction of the microcirculatory function and thus of relevant substrates for energy metabolism, and a poor ATP yield in glycolysis which becomes more pronounced in therapeutic hyperthermia[22]. Measurement of microregional ATP concentration distributions by single-photon imaging and quantitative bioluminescence confirmed these data[9].

As a result of intensified ATP degradation, an accumulation of purine catabolites is to be expected, together with formation of protons at several stages during degradation to the final product, uric acid[13, 15]. Proton formation can in turn contribute to the development of heat-induced acidosis. Furthermore, oxidation of hypoxanthine and xanthine may result in the formation of active oxygen species[13, 15], which may lead to DNA damage, lipid peroxidation and protein denaturation[17, 26], thus also contributing to heat-induced cytotoxicity. To test this hypothesis, experiments were performed on subcutaneously growing experimental tumors exposed to localized hyperthermia at clinically relevant tumor tissue temperatures[11, 16, 23, 24].

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Bioenergetic status of solid tumors upon localized heating

Several comprehensive studies of the effect of therapeutic heating on tumor energy metabolism have been performed[24]. The results of these investigations are qualitatively quite similar, with only small differences being found accountable to the heat dose, the heating technique, the tumor volumes employed and the use or absence of general anesthesia.

In vivo ^{31}P nuclear magnetic resonance (NMR) spectroscopy was used to monitor the bioenergetic status, pH_{NMR} and membrane phospholipid turnover in subcutaneously growing murine fibrosarcomas and mammary carcinomas treated at 43.5 °C for 15, 30 or 60 min. Experiments were performed on conscious mice with biologically relevant tumor volumes[24]. The study focussed on acute heat-induced bioenergetic changes (up to 7 hours post-heating). ^{31}P NMR spectra of both murine tumors were characterized by relatively high pre-treatment levels of phosphomonoesters (PME), inorganic phosphate (P_i) and nucleoside triphosphates (NTP), and lower levels of phosphodiesters, phosphocreatine (PCr) and diphosphodiesters. Following hyperthermia, NTP and PCr levels decreased. This drop was accompanied by a prompt and substantial increase in

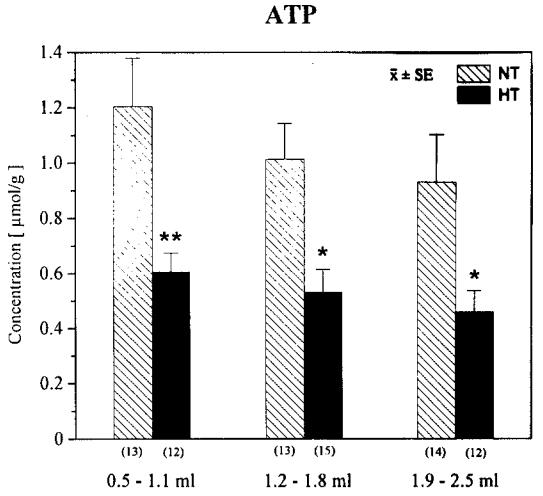

Figure 1. ATP concentrations in control (left columns) and heated (44 °C/60 min) tumors (right columns) of three different volume classes (DS-sarcomas heated with water-filtered infrared-A-radiation). Values are means ±SE (* $p < 0.05$, ** $p < 0.01$). NT: normothermia, HT: hyperthermia. Number of tumors investigated in parentheses.

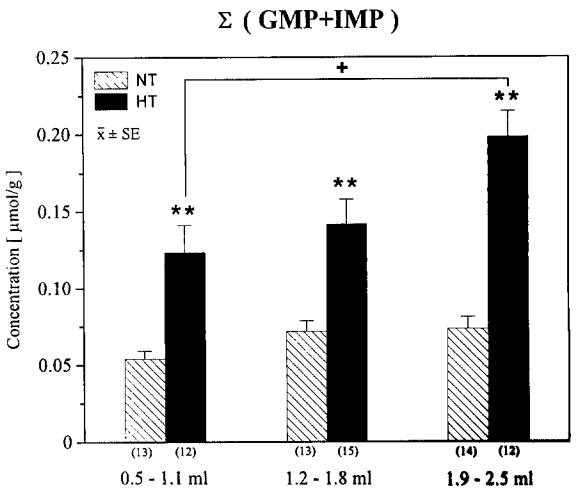

Figure 2. Sum of guanosine (GMP) and inosine (IMP) monophosphate levels in control (left columns) and heated (44 °C/60 min) tumors (right columns) of three different volume classes (DS-sarcomas heated with water-filtered infrared-A-radiation). Values are means ±SE (+ $p < 0.05$, ** $p < 0.01$). NT: normothermia, HT: hyperthermia. Number of tumors investigated in parentheses.

P_i. After heating for 15 min, the limited spectral changes observed for the high-energy phosphates were nullified within 7 hours, whereas P_i remained significantly elevated. Metabolic ratios (PCr/P_i and NTP/P_i) decreased after heating and did not recover thereafter. Upon longer heat exposure times (30 and 60 min) the high-energy phosphates, PCr/P_i and NTP/P_i all decreased in a dose-dependent manner and remained at the respective lower levels[24].

The remaining NTP resonances after 'subcurative' heat doses showed chemical shifts suggesting that high concentrations of nucleoside diphosphates were present[24]. Concomitantly, PME levels often increased, consistent with the accumulation of glycolytic intermediates and perhaps of nucleoside monophosphates. As a rule, all of these changes can reverse within 24–36 hours after hyperthermia.

Analysis of tumor bioenergetic status using high-pressure liquid chromatography (HPLC) and acid tissue extracts

In rat DS-sarcomas, global concentrations of ATP (HPLC), P_i and PCr (enzymatic tests) were measured before and immediately after 44 °C-hyperthermia for 60 min using water-filtered infrared A radiation[23] or saline-bath heating[11,16]. Mean pre-heating ATP levels were almost independent of tumor volume and ranged from 0.8 to 1.6 µmol/g. Upon hyperthermia, global ATP concentrations significantly decreased (fig. 1) and were accompanied by a significant increase in the fraction of tumor tissue exhibiting cellular damage[16]. The extent of these heat-induced changes was not related to tumor size.

The ATP decline observed upon hyperthermia is most probably due to several mechanisms. Most relevant in this context are (1) an increased ATP turnover rate (intensified ATP hydrolysis) during heating; (2) a poorer ATP yield (on a molar basis) during hyperthermia because there is a shift from oxidative glucose breakdown to glycolysis; (3) a restriction of the microcirculatory function and thus of relevant substrates for energy metabolism (in some tumor lines); and hypothetically (4) an inhibition of the adenylate kinase reaction due to tissue acidosis (a so far unproven notion). Mean P_i concentrations increased (from 6.5 ± 0.9 to 10.0 ± 1.0 µmol/g), and PCr levels decreased (from 1.2 ± 0.1 to 0.2 ± 0.1 µmol/g), with increasing tumor size. Upon hyperthermia, the former tended to increase and the latter to decrease. The extent of these heat-induced changes was not related to tumor size, a finding also observed with ATP.

Levels of adenosine diphosphate ADP (0.4 ± 0.04 µmol/g) and of adenosine monophosphate AMP (0.3 ± 0.04 µmol/g) as well as adenylate energy charge AEC (0.75 ± 0.03) remained relatively constant with increasing tumor volume. Following therapeutic hyperthermia, AEC decreased significantly, ADP dropped slightly, whereas no changes were observed in AMP concentrations.

Purine catabolites in solid tumors upon localized heat treatment

Purine catabolites were determined by gradient ion-pair, reversed-phase HPLC at 254 nm. Following termination of heat treatment, tumors were rapidly excised,

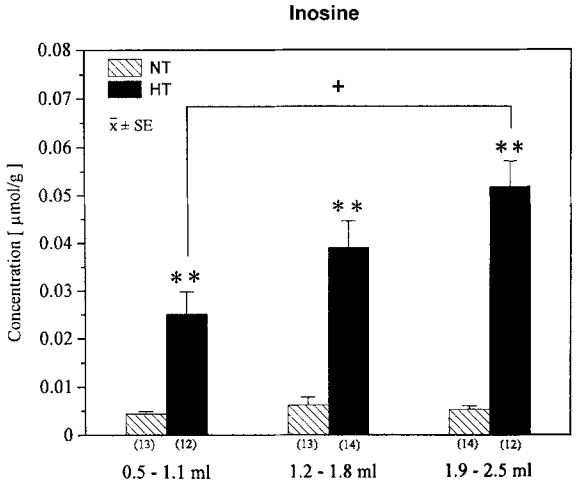

Figure 3. Tumor volume-dependent increase in inosine concentrations in rat DS-sarcomas following localized 44 °C-hyperthermia for 60 min. NT: normothermia, HT: hyperthermia ($+p < 0.05$, $** p < 0.01$). Number of tumors investigated are given in parentheses.

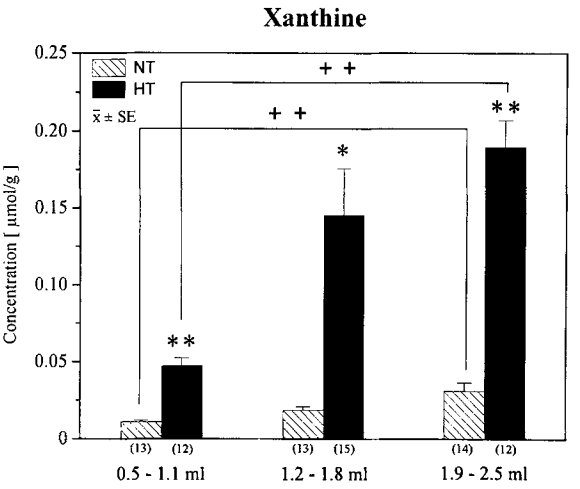

Figure 5. Tumor volume-dependent increase in xanthine concentrations in rat DS-sarcomas upon 44°C-hyperthermia for 60 min. NT: normothermia, HT: hyperthermia ($* p < 0.05$, $++$ and $** p < 0.01$). Number of tumors investigated are given in brackets.

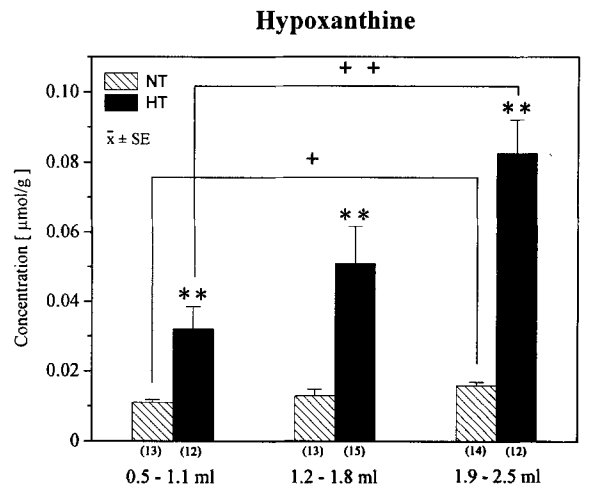

Figure 4. Tumor volume-dependent increase in hypoxanthine levels in rat DS-sarcomas upon 44 °C-hyperthermia for 60 min. NT: normothermia, HT: hyperthermia ($+p < 0.05$, $++$ and $** p < 0.01$). Number of tumors investigated are given in parentheses.

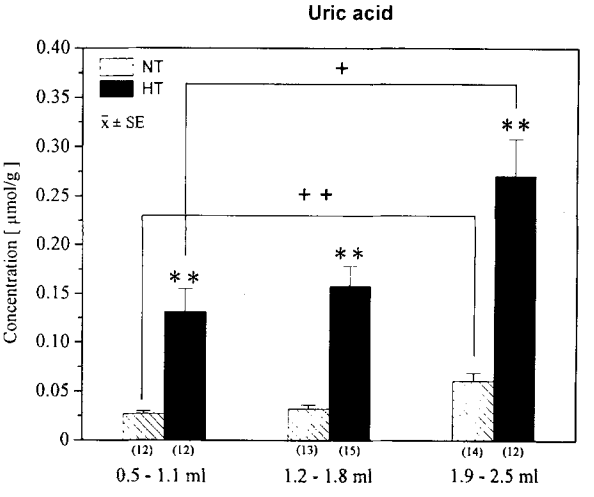

Figure 6. Tumor volume-dependent increase in uric acid levels in rat DS-sarcomas upon 44 °C-hyperthermia for 60 min. NT: normothermia, HT: hyperthermia ($* p < 0.05$, $++$ and $** p < 0.01$). Number of tumors investigated are given in parentheses.

frozen in liquid N_2 and freeze-dried. Acid-extracted, neutralized tissue samples were analyzed using the peak heights and considering external standards of known concentrations[9,11]. In tumors the concentrations of purine nucleotides were severalfold higher than the levels of purine bases, necessitating a range shift in absorption sensitivity (change from 0.10 to 0.35 Aufs after 28 min).

The following purine metabolites were assessed: nucleotides: ATP, ADP, AMP, GTP, guanosine diphosphate (GDP), Σ[IMP + GMP] (limit of detection: <10 nmol/g); nucleosides: inosine, guanosine, adenosine (limit of detection: <2 nmol/g); purine bases: hypoxanthine, xanthine, adenine (limit of detection: <1 nmol/g); and uric acid (limit of detection: <10 nmol/g).

In hyperthermia experiments a tumor volume-dependent, significant increase in the levels of the following catabolites was observed: Σ[IMP + GMP] (volume-dependent increase by a factor of 2.0–2.7; fig. 2); inosine (INO, volume-dependent increase by a factor of ~10; fig. 3); hypoxanthine (HX, volume-dependent increase by a factor of 3.0–5.4; fig. 4); xanthine (X, volume-dependent increase by a factor of 5.1–7.8; fig. 5); and uric acid (UA, volume-dependent increase by a factor of ~4.5; fig. 6).

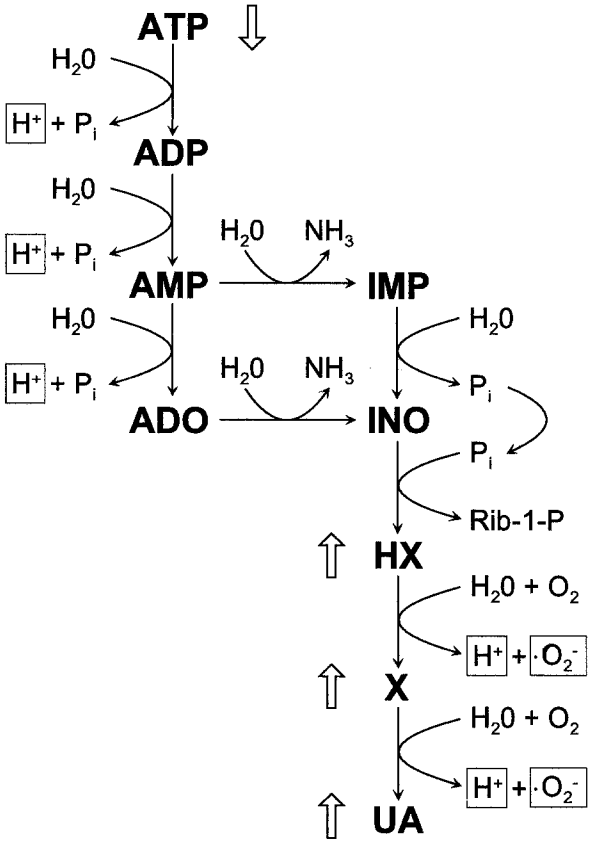

Figure 7. Schematic representation of ATP catabolism in solid tumors upon therapeutic hyperthermia.

Conclusions

The accumulation of purine catabolites in tumor tissue upon localized heat treatment, coinciding with a significant ATP (and a slight GTP) depletion, is most probably the result of intensified ATP hydrolysis during hyperthermia. During hydrolysis of 1 mole of ATP there is a formal production of four to five protons (fig. 7) which may in turn, at least partially, contribute to the heat-induced acidosis that is known – as with energy depletion – to greatly enhance the thermosensitivity of cancer cells[6,14] and to inhibit the development of thermotolerance[12].

Additionally, oxidation of hypoxanthine and xanthine results in the formation of oxygen-activated species[17,26]. Superoxide radical anions and superoxide molecules can react, forming the highly active hydroxyl radical (Haber-Weiss reaction). All reactive oxygen species can lead to lipid peroxidation, protein denaturation and DNA damage, thus contributing to heat-induced cytotoxicity.

There is circumstantial evidence for the postulated action of reactive oxygen species in therapeutic hyperthermia:
– increase in lipid peroxidation in VX2 tumors and decrease of the antitumor effect of heat upon administration of free radical scavengers (catalase, superoxide dismutase, DMSO)[25];
– increase in lipid peroxidation upon intensified purine catabolism during hyperthermic (42–42.5 °C) perfusion of human livers with cancer[18], and in rat or guinea pig liver resulting in peroxidative damage[2];
– increase in lipid peroxide levels upon hyperthermia (43 °C/60 min) in C3H mouse mammary tumors[1];
– the generation during hyperthermia of oxygen radicals, which overrides the rate of superoxide radical elimination by superoxide dismutase and leads to a loss of cellular protective capacity and thus to cytotoxic effects[8]; and
– constriction of tumor arterioles upon localized hyperthermia through inactivation of the vasodilator NO by reactive oxygen species[3].

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