Exp Oncol 2005
27, 1, 61–64

Experimental ONCOLOGY

WHOLE BODY HYPERTHERMIA SUPPLEMENTED WITH UROTROPIN IN THE TREATMENT OF MALIGNANT TUMORS

Reiman S. Ismail-zade
Oncology Department, Belarusian Center for Pediatric Oncology and Hematology, P.O. Lesnoy, Minsk 223040, Belarus

***Aim*: To evaluate the effect of urotropin on the tolerance of an extreme whole body hyperthermia (WBH) regime (44.5–45.0 °C for 60 min) in rats, and then its application to pediatric patients with advanced cancer during WBH sessions (42.5–43.0 °C). *Methods*: In experiments nonbred rats bearing Sarcoma 45 (Sa 45) were subjected to severe WBH with and without urotropin. Tolerance of WBH as well as tumor growth and survival of animals were monitored. Extreme WBH sessions (42.5–43.0 °C) + urotropin were used in multimodal treatment of 13 children with advanced, refractory or recurrent malignant tumors (42 procedures in total). *Results:* Our experiments showed 5-fold increase in rat survival during the first two days following application of extreme WBH due to the additional administration of urotropin. This regime demonstrated a significant inhibitory effect upon the growth of Sa 45. In children with progressive malignant tumors we achieved extreme WBH regimes (up to 43.2 °C) using urotropin without additional toxicity and with 69% overall response rate. *Conclusion:* Extreme WBH (42.5–43.0 °C) could be used in the management of advanced and refractory cancer. Urotropin may play a favorable role during the procedures of extreme WBH, decreasing thermal damage to the body. Lymphocyte collection by lymphopheresis before WBH session and its reinfusion may be one of the progressive approaches for minimizing lymphocyte apoptosis.**
***Key Words:* extreme WBH, Sarcoma 45, urotropin, cancer in children.**

Whole body hyperthermia (WBH), as a treatment modality, shows promising results in the management of advanced and refractory cancer in combination with chemotherapy [1–4]. Techniques have been dramatically improved in the last 20 years. Progress in improving the safety of the therapy depends on technical advances and appropriate anesthetic management. In general, in a standard WBH session systemic temperatures of 41–42 °C could be achieved and maintained for 60–120 min. If body temperature is raised above 42.5 °C and this regime maintained at least for 10 min than we could consider it as an extreme WBH. The issue is whether extreme WBH is to the benefit or detriment of the patient. On one hand higher temperature is better for tumor eradication, but on the other hand, tumor killing regimes in WBH (> 42.5 °C) are not safe and inhibit host immunocompetence [5].

At the XXIV International Congress on Clinical Hyperthermia (Rome, 2001) Souvernev et al. [6] presented results of the clinical application of severe WBH (43–44 °C) given for few minutes in the treatment to adult patients with cancer and viral infection. Urotropin (cystamine, hexametilentetramine) has been used for preventing thermal proteolysis in critical body temperature. It is known that urotropin was at first proposed by G.K. Gleim in 1997 for this purpose [7]. Increasing the peak temperature up to 43–43.5 °C and maintaining it for 10–15 min during a WBH session, with a temperature plateau of 42 °C, could be effective for antineoplastic activity. At the same time the attention should be concentrated on the safety of the procedure and possible alteration of the lymphocyte subpopulations due to apoptosis.

In the present study we evaluated the effect of urotropin on the tolerance of severe WBH regimes in rats bearing Sa 45 and in pediatric patients with advanced cancer.

Received: December 10, 2004.
Correspondence: E-mail: reiman1955@mail.ru
Abbreviations used: CT — chemotherapy; ES — Ewing's sarcoma; GCT — germ-cell tumor; HB — hepatoblastoma; HCC — hepatocellular carcinoma; HF EM — high-frequency electro-magnetic; IL-2 — interleukin-2; LAK- lymphokine-activated killers; NPC — nasopharyngeal carcinoma; RCC — renal cell carcinoma; WBH — whole body hyperthermia; WT — Wilms tumor.

MATERIALS AND METHODS

Experiments were carried out at the N.N. Alexandrov Research Institute of Oncology & Medical Radiology (Minsk, Belarus). 30 female white nonbred rats, aged 2–3 months and weighing 140–160 g were used. Sarcoma 45 (Sa 45) cells were obtained from Blokhin's Research Cancer Center (Moscow, Russia). Tumor cell inoculation was performed by subcutaneous injection of 0.5 ml of tumor cell suspension (1 x 10^6 cells) into the lower part of the left femoral region. Five days later, the rats with proven subcutaneous tumor growth were subjected to the following treatment (10 rats in each group); a) control group treated with WBH 42.5–43.0 °C for 1 h, b) a group treated with WBH 44.5–45.0 °C for 1 h, c) a group treated with WBH 44.5–45.0 °C for 1 h + urotropin. Water-bath heating was used for inducing controlled WBH in rats. Urotropin (40% solution) was injected twice (0.2 ml/100 g before WBH and in a half dose — after WBH). Animal experiments were performed according to the Rules of Ethic Committee of the N.N. Alexandrov Research Institute of Oncology & Medical Radiology.

Prior to each manipulation, animals were anesthetized with droperidol and fentanil (respectively 5 mg/kg and 0.05 mg/kg of body weight intramuscularly).

In vivo antitumor activity was evaluated and the following parameters were evaluated:

• tumor size was determined with calipers by measuring three orthogonal diameters (groups a,b,c) of the tumor mass twice per week until day 33 following tumor inoculation; tumor volume in cm^3 was calculated according to Schrek's formula: $V = (a \cdot b \cdot c) \cdot 0.52$;

• antitumor effect of the treatment was determined as inhibition of tumor growth rate in comparison with untreated control; tumor growth inhibition ratio (TGIR, in%) was calculated by formula:

$$TGIR = \frac{V_{mn\ (con)} - V_{mn\ (exp)}}{V_{mn\ (con)}} \times 100\%;$$

where $V_{mn\ (con)}$ and $V_{mn\ (exp)}$ are the volume of the tumor in the control and experimental group of animals, respectively;

• mean survival time of tumor–bearing rats die up to the day 120 of observation (in days) was registered;

• percentage of cured rats — proportion of rats without detectable tumor at the day 120 of observation after tumor cell inoculation as compared to total number of animals in groups.

Statistical analysis. Values given in this study are mean + SEM. Unpaired Student's tests were used for all calculations

In clinical practice WBH sessions were administered using high–frequency electro–magnetic (HF EM) installation (Yachta–5, Russia), under general anesthesia with orotracheal intubation and mechanical ventilation. HF EM field with frequency of 13.56 MHz was created by a coplanar capacity type applicator positioned under distilled water filled bolus (mattress) that a patient was lying on. The HF EM energy being released directly in the deep tissues ensures effective whole body heating to the required therapeutic temperature up to 43 °C, whereas the skin temperature can be maintained as low as 39–40.5 °C [8]. Rectal and esophagus temperatures were monitored at 10 min intervals using thermistors. Rectal temperature regimen maintained 41.8–42.5 °C for 2 h with peak temperature up to 43.3 °C for 10–15 min. Urotropin was given i.v. (40–80 mg/kg) twice (at the peak of temperature and end of procedure) to prevent thermal tissue proteolysis. The blood glucose level of 20–22 mmol/L was achieved and maintained throughout the procedure. Cranial parts of the body were cooled with air conditioning system after achieving a temperature plateau of 42 °C. The blood pressure, heart rate and respiration were kept under control within age–related ranges. In some cases sinus tachycardia required the use of antiarhythmics. Urinary output as well as blood electrolyte levels and acid–base status were continuously monitored. In order to maintain mean arterial pressure of at least 60 mm/Hg and diuresis of approximately 1 ml/kg, fluid volume substitution was performed by infusion of crystalloid and colloidal solutions. WBH was accompanied by appropriate anesthetic management and monitoring, therefore did not lead to any serious or sustained organ disfunction, and was regarded as a safe therapy. A typical extreme WBH procedure lasted 3–3.5 h, including 40–45 min of heating to reach target temperature and 35–40 min for cooling phase. In total 42 WBH sessions were performed in 13 patients. Changes of cellular immunity (before and 1 day after WBH procedure) were measured by FACscan Cytometry (Becton Dickinson). Apoptosis rates of lymphocytes were determined by staining with Annexin V.

RESULTS AND DISCUSSION

Experimental data (Table 1–3 and Fig. 1) show that extreme WBH (44.5–45.0 °C — 60 min) with urotropin results in delayed death of animals in 50% of cases as well as significantly higher rectal temperatures during the procedure. Such a regime of extreme WBH (44.5–45.0 °C — 60 min) without urotropin was not tolerated by the rats, and 90% of them died after the procedure. It should be underlined that rectal temperature in these animals was 1.62 °C lower at the end of procedure in comparison with the same temperature regime with urotropin. These results suggest that urotropin may have an effect on the thermoregulation mechanisms of animals. Besides, as shown in Table 2, the application of extreme WBH (44.5–45.0 °C — 60 min) + urotropin in rats demonstrated significant inhibitory effects upon the subcutaneous growth of Sa 45 as compared to the usual regime of 42–43.0 °C. Urotropin may play a favourable role in extreme WBH, decreasing thermal damage to the body. Being of nonpeptide composition with a low molecular weight it works even in the critically high temperatures, whereas all peptide antiproteolitics stop working due to inactivation.

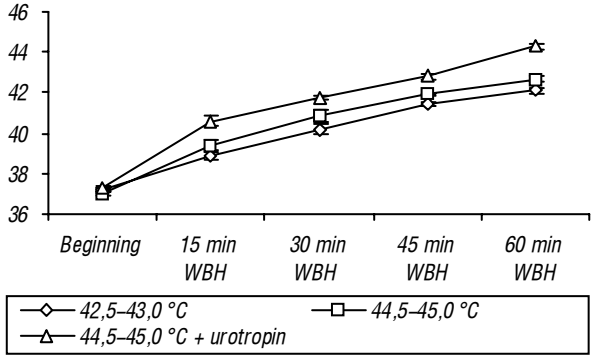

Fig. 1. Rectal temperature in rats bearing Sa 45 during WBH procedure (60 min) in different regimes

Table 1. *Survival of animals after 60 min session of WBH with different temperature regimes*

Temperature regimes	Number of animals										
	Died in terms of hours										Survived
	1	2	4	6	8	10	12	22	24	48	
42.5–43.0 °C	0	0	0	0	0	0	0	0	0	0	10
44.5–45.0 °C	2	3	1	0	0	0	0	1	1	1	1
44.5–45.0 °C + urotropin	0	0	0	0	0	0	0	2	3	0	5

Table 2. *Tumor growth inhibition ratio in rats bearing Sa 45 in comparison with control group after WBH*

Treatment characteristics	Tumor growth inhibition ratio (days)							
	8	12	15	19	22	26	29	33
WBH 42.5–43.0 °C – control	23.6	18.8	26.0	26.8	23.2	22.6	20.4	24.1
WBH 44.5–45.0 °C + urotropin	45.4	55.4	58.3	60.2	53.0	42.2	36.4	36.8

Table 3. *Mean survival time (days) and percentage of cured rats bearing Sa 45 after WBH*

№	Groups		
	Control	WBH 42.5–43.0 °C	WBH 44.5–45.0 °C + urotropin
x±	34.4	37.3	37.3
Sx	1.62	2.74	3.01
Cure rate	—	—	10%

Table 4. *Clinical data on children with advanced malignances subjected to severe WBH*

Pt No	Sex	Age	Histology	TNM	Tumor affected sites	Number of WBH session	Temperature (°C)	Chemotherapy	Tumor response	Salvage or adjuvant therapy
1	M	13	NPC	$T_3N_2M_1$	Nasopharynx, servical lymphnodes and multi– skeleton metastasis	2	42.5	Carboplatin + VP–16	SD	Salvage
2	M	10	Undifferentiated cancer	$T_3N_2M_1$	Rectum and multi–lung metastasis	4	42.5–42.7	Carboplatin + VP–16	CR	Salvage
3	F	10	GCT	$T_2cN_0M_0$	Both ovary and pelvioperitoneal metastasis	1	43.0	IFO + VP–16	PR	Salvage
4	F	7	WT	$R_1TN_1M_1$	Retroperitoneal area, liver and multi lung metastasis	3	42.5	Carboplatin + VP–16 IFO + VP–16	PR	Salvage
5	F	6	Askin tumor	$T_3N_1M_1$	Right thoracopulmonal area + mediastinal & multi– lung metastasis	2	42.5	Carboplatin + VP–16 Carboplatin + ADR	SD	Salvage
6	M	7	Askin tumor	$R_1T_1N_0M_1$	Right thoracopulmonal area & pleural metastasis	4	42.5–43.0	Carboplatin + VP–16 IFO + VP–16 Carboplatin + IFO	PR	Adjuvant
7	M	13	RCC	$T_4N_2M_1$	Right kidney + retroperitoneal area and multi–lung metastasis	5	42.5–43.2	ADR + Intron A Carboplatin	SD	Salvage
8	F	12	HCC	$T_3N_0M_0$	Right lobe with invasion to the left lobe	5	42.5–43.0	Carboplatin 5–FU + Intron A	PR	Salvage
9	F	13	ES	$R_1TN_0M_1$	Left paraspinal area with lung metastasis	3	42.5–43.0	Carboplatin + VP–16 IFO + VP–16 Carboplatin + IFO	SD	Adjuvant
10	M	10	HB		Both lobes & lung metastasis	3	42.5–43.2	5–FU + Intron A	PR	Salvage
11	F	9	HCC	$R_1T_3N_0M_0$	Right lobe	3	42.5–43.0	5–FU + Intron A	PR	Salvage
12	M	16	GCT		Left testis with retroperitoneal and multi–lung metastasis	2	42.5	Bleomycin + VP–16	PR	Adjuvant
13	F	12	RCC	$T_3N_2M_1$	Left kidney + retroperitoneal and mediastinal lymph nodes metastasis	5	42.5–43	ADR + Intron A	PR	Adjuvant

Between 2001 and 2004, 13 children with advanced, refractory or recurrent malignant tumors were treated with extreme WBH (42.5–43.2 °C) and chemotherapy (CT). Consent of parents for treatment had been obtained in all cases. The median age was 10.6 years (range 7–16 years). Patients' characteristics, therapy and response to therapy are shown in Table 4.

In 9 children we used WBH as a salvage therapy in nonresponders and early relapsed cases. The majority of patients had been treated according to accepted European protocols. Two of them had hepatocellular carcinomas (HCC), others had Askin tumor, renal cell carcinoma (RCC), hepatoblastoma (HB), Wilms tumor (WT), germ–cell tumor (GCT), nasopharyngeal carcinoma (NPC) and undifferentiated carcinoma.

In four children with high risk tumors, extreme WBH was administered as an adjuvant therapy to the standard protocol chemotherapy cycles. These were patients with Askin tumor, Ewing's sarcoma (ES), RCC and GCT.

All patients tolerated WBH procedures without any serious complications. There was no major (WHO grade 3–4) acute toxicity related to thermochemotherapy. Occasionally we observed superficial skin burns which did not require any surgical managment. Evaluation of treatment–related toxicity and monitoring cardio–vascular system, kidney function, liver enzymes and other were carried out carefully before and after every procedure, as well as after 6–12 months following treatment.

The response to the extreme WBH with chemotherapy was evaluated in all patients. There was one complete response (CR), eight partial responses (PR) and four stable disease (SD), for an overall response rate of 69.23%.

We published earlier [9] that the immunological consequences of combined therapy, including WBH, chemotherapy and interleukin–2 (IL–2, Roncoleukin), in children with advanced tumor, are the increased lymphocyte apoptosis and lymphopenia. It is obvious from our results that lymphopenia after WBH is reversable and application of cytokines does not prevent it (Fig. 2). We have studied the possibility to clarify if lymphocyte apoptosis is reversal after multisessional WBH application during combined therapy of children with cancer. As shown in Fig. 3, WBH sessions (including all procedures in 13 patients with urotropin and 7 cases without) evidently resulted in increased level of apoptotic lymphocytes in peripheral blood of patients. The same results were achieved by some authors [11]. There are different approaches to minimize harmful effects of WBH. Cytokine application may be useful because it is known that it has a beneficial effect upon lymphocytes undergoing stress–induced apoptosis [10].

For minimizing lymphocyte apoptosis during extreme WBH we proposed lymphocyte collection by lymphopheresis before WBH session and then reinfusion at the end of WBH when temperature drops to 40–40.5 °C. This way

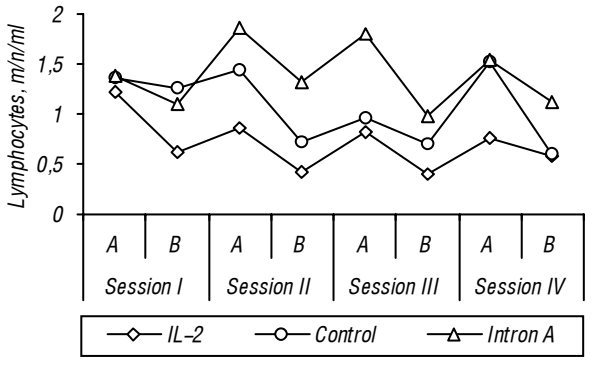

Fig. 2. Absolute number of lymphocytes in peripherial blood before (*A*) and after (*B*) WBH with CT and additional administration of cytokines (IL–2 and Intron A)

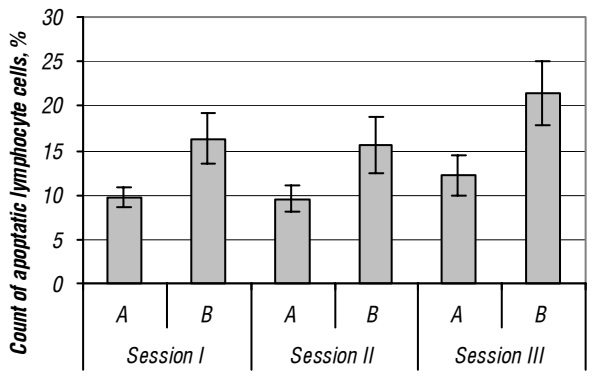

Fig. 3. Level of apoptotic lymphocytes in peripherial blood of patient before (*A*) and after (*B*) WBH

we could save at least 1–2 · 10^8 lymphocytes [12]. Collected lymphocytes may be incubated in special medium with IL–2 for achieving lymphokine–activated killers (LAK) cells and infused as well as lymphocytes.

In conclusion, we have shown on tumor–bearing rats that urotropin could play a protective role from thermal damage in extreme WBH procedure. In limited groups of children with progressive and relapsed malignant tumors we achieved extreme WBH regimes (up to 43.2 °C in rectum) using urotropin. Overall response rate achieved was 69.2% in a poor prognostic group of patients. Extreme WBH regimes are favourable for tumor eradication, but on the other hand they may inhibit host immunocompetence by inducing apoptosis in a subpopulation of blood lymphocytes. Lymphocyte collection by lymphopheresis before WBH session and its reinfusion may be one of the progressive approaches for minimizing lymphocyte apoptosis.

REFERENCES

1. **Ardenne M.** Systemische Krebs-Mehrschritt-Therapie. Stuttgart: Hippokrates-Verlag, 1997; 302 p.

2. **Krasny SA, Mavrichev AS, Zhavrid EA, Sukonko OG, Fradkin SZ, Polyakov SL.** Whole body hyperthermia in the management of renal carcinoma patients with metastases in regional lymph nodes. Exp Oncol 1998; **20**: 66–70.

3. **Ismail-zade RS, Zhavrid EA, Potapnev MP.** Whole body hyperthermia in pediatric solid tumor treatment. Ann Oncol 2002: **13**: 49.

4. **Ismail-zade RS, Buglova SE, Potapnev MP, Sachivko NV, Semenov AS, Rudko VS, Shman TV, Zhavrid EA.** Salvage thermochemotherapy in managment of advanced refractory malignant tumors in children. Voprosy Oncologii 2002; **48**: 351–5 (in Russian).

5. **Fritz KL, Koziol S, Fabian DF, Lefor AT.** Tumor necrosis factor alpha mediates the antitumor effect of combined interleukin-2 and whole body hyperthermia. J Surg Res 1996; **60**: 55–60.

6. **Souvernev AV, Vereschagin EI, Kinzt DN, Pisarev AS, Tulenev PS, Vereschagin EI, Efremov AV.** Clinical effects of whole body severe hyperthermia (43.5–44 °C). Abstracts of XXIV International Congress on Clinical Hyperthermia. Rome, September 24–29, 2001.

7. **Souvernev AV.** Method of whole body hyperthermia. Patent of Russian Fedaration № 2126667 from May 5, 1998.

8. **Mazokhin VN, Kolmakov DN, Lucheyov NA, Gelvich EA, Troshin II.** A HF EM installation allowing simultaneous whole body and deep local EM hyperthermia. Int J Hyperthermia 2000; **16**: 287–90.

9. **Buglova SE, Ismail-zade RS, Savitskiy VP, Zhavrid EA, Belevtsev MV, Potapnev MP.** Immunomodulatory effect of thermochemotherapy combined with Roncoleukin in therapy of advanced pediatric malignant tumors. Klin Immunologiya 2003; **24**: 107–10 (in Russian).

10. **Potapnev MP.** Immune system cell apoptosis and its modulation by cytokines. Immunologiya 2002; **23**: 237–43 (in Russian).

11. **Dieing A, Ahler O, Kerner T, Wust P, Felix R, Loffel J, Riess H, Hildebrant B.** Whole body hyperthermia induces apoptosis in subpopulation of blood lymphocytes. Immunobiology 2003; **207**: 265–73.

12. **Ismail-zade RS, Zhavrid EA, Potapnev MP, Mavrichev AS.** Method of the treatment malignant tumours. Patent of the Republic of Belarus № 6066 from December 12, 2003.

ОБЩАЯ ГИПЕРТЕРМИЯ В СОЧЕТАНИИ С УРОТРОПИНОМ В ЛЕЧЕНИИ ЗЛОКАЧЕСТВЕННЫХ ОПУХОЛЕЙ

Целью **экспериментальной части исследования являлось изучение влияния уротропина (гексаметилентетрамина) на переносимость сверхжестких режимов общей гипертермии (ОГ) и оценка их противоопухолевого эффекта у крыс. Клинически исследовалась возможность применения и эффективность ОГ в жестком режиме в комбинации с уротропином при лечении детей со злокачественными опухолями поздних стадий.** ***Методы*****: сверхжесткий режим ОГ (44,5–45,0 °С, 60 мин) был применен к 30 белым беспородным крысам с привитой саркомой 45. В клинике сеансы ОГ (42,5–43,0 °С) проводились на установке "Эмона" с помощью электромагнитного излучения (13,56 МГц).** ***Результаты*****: в ходе эксперимента уротропин повышал переносимость высоких температур и сделал возможным применение к крысам сверхжестких режимов ОГ с выраженным противоопухолевым эффектом. В клинических условиях комбинация ОГ + уротропин использовалась в многокомпонентном лечении 13 детей с различными злокачественными опухолями поздних стадий. Всего проведено 43 сеанса, и при удовлетворительной переносимости лечения полная или частичная регрессия опухолевых очагов отмечена у 69% больных.** ***Выводы*****: уротропин повышает переносимость жестких режимов ОГ, уменьшая последствия теплового воздействия на организм. Проведение ОГ в более высоком температурном режиме (42,5–43,0 °С) усиливает повреждение опухоли. Однако ОГ может усилить и апоптотическую гибель лимфоцитов. Лимфоферез перед сеансом ОГ и реинфузия аутолимфоцитарной массы после него может быть одним из новых подходов, уменьшающих негативное влияние лечения на лимфоциты.**

Ключевые слова**: общая гипертермия, уротропин, саркома 45, крысы, опухоли у детей.**