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Original

Radioiodine therapy temporarily increases circulating endothelial cells and decreases circulating endothelial progenitor cells

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Abstract

BACKGROUND: Radiotherapy can cause vascular injury. No data on radioiodine therapy and vascular damage are available.

MATERIAL AND METHODS: We examined the number of circulating endothelial cells (CEC) and circulating endothelial progenitor cells (CEPC) before therapy 1, 2, 3 and 5 days as well as 1, 2, 3, 4, 6, 8, 10, and 12 weeks after therapy with ^{131}I at doses ranging from 5–200 mCi. The individual number of CEC and CEPC is associated with the presence of risk factors.

RESULTS: Irrespective of prevalues, CEC exhibited a significant dose-dependent temporary increase reaching the maximum in weeks 1 and 2. In contrast, CEPC show a decrease at the same time.

CONCLUSIONS: These results indicate that ^{131}I-therapy induces a dose-dependent radiation injury at the vascular wall level enhancing endothelial desquamation and reducing reendothelialization and thereby a proatherogenic stage. The clinical consequence of these findings still needs to be assessed.

Key words: radioiodine therapy, thyroid cancer, circulating endothelial cells, radiation injury

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Introduction

The value of radioiodine therapy in hyperthyroidism and thyroid cancer is well established. Side effects even after repeated administration are rare except sialadenitis [1]. Recently, signs of temporarily impaired spermatogenesis have been reported in patients treated for differentiated thyroid cancer [2]. Furthermore, radioiodine therapy induces LDL-modification rendering the molecule more atherogenic [3]. *In–vivo* oxidation injury has been assessed measuring the isoprostane 8-epi-$PGF_{2\alpha}$ in saliva [4] as well as in blood and urine [5]. Data on vascular injury are not yet available. It has been shown, that irradiation may cause severe endothelial damage. An increased number of CEC have been detected in association with vascular injury. They negatively correlate with platelet survival and represent proof of serious damage to the vascular tree. More recently, CEPC finally differentiating into mature endothelial cells have been discovered as an important measure for (re-)endothelialization. This study was aimed at assessing the kinetics of circulating endothelial (progenitor) cells.

Materials and methods

42 patients undergoing radioiodine therapy (for characteristics see Tab. 1) for hyperthyroidism or differentiated thyroid cancer were investigated for the determination of CEC and CEPC.

A prevalue was obtained the morning before radioiodine administration, and thereafter at days 1, 2, 3 and 5 as well as 1, 2, 3, 4, 6, 8, 10 and 12 weeks after therapy in the morning after an overnight fast.

The determination of CEC

4.5 ml of blood were drawn from the cubital vein into 0.5 ml of 3.8% sodium citrate as an anticoagulant. After sedimentation for 10 minutes at room temperature (22°C) platelet rich plasma (PRP) was prepared by centrifugation for 10 minutes at 150 × g. 1 ml of PRP was transferred into an aggregometer and aggrega-

Table 1. Patients characteristics (therapeutic dose and risk factors)

group	5 mCi	20 mCi	80 mCi	200 mCi
N	17	11	6	8
M	8	4	3	3
F	9	7	3	5
Age	47–67	44–65	45–71	53–68
SM	5	3	2	2
HY	1	2	1	1
DM	1	3	1	2
HLP	5	4	3	3

SM — smokers; HY — hypertension; DM — diabetes mellitus; HLP — hyperlipoproteinemia

Table 2. Influence of risk factors

	CEC	CEPC
OR	3.2 ± 1.7	314 ± 21
SM	24.6 ± 9.6*	206 ± 30*
HY	5.8 ± 2.2	247 ± 24
DM	8.6 ± 4.2	202 ± 30*
HLP	19.5 ± 6.6*	196 ± 27*

values in X ± SD; *) $p < 0.01$ *vs.* OR (no risk factor); CEC — circulating endothelial cells; CEPC — circulating endotheliac progenitor cells; for other abbreviations see Table 1

tion was induced with a high dose (50 mM in 100 ml) of ADP. Platelet aggregates were removed by a further centrifugation step. The fragments of CEC were pelleted by a final centrifugation for 10 minutes at 1000 × g. This pellet was dissolved in Plaxan and counted in a Bürker-Türk chamber. The number of CEC is given per ml as is conventional in expressing blood cell counts. The inter-assay variation amounts to 2.4 ± 0.5 (> 20 CEC/ml) and 5.7 ± 2.6 (< 10 CEC/ml). The intraassay variation is 1.9 ± 0.4% (> 20 CEC/ml) and 3.7 ± 1.4% (< 10 CEC/ml), respectively.

The determination of CEPC

Venous blood was collected using heparin as an anticoagulant. Within 2 hours of the blood collection, the peripheral blood mononuclear cells were isolated by Ficoll-Paque (Pharmacia Biotech LKB, Vienna, Austria) density-gradient centrifugation. The recovered cells were washed twice in phosphate buffered saline and twice in RPMI 1640 medium containing 10% fetal calf serum (including 100 U/ml of penicillin and 100 mg/ml of streptomycin). Fluorescence activated cell sorting (FACS) analysis revealed a purity > 90% and a viability > 99% (determined via trypan blue exclusion). Thereby isolated cells were resuspended in a growth medium and plated on fibronectin coated wells (5.10^6/well). After 48 hours the non-adherent cells were collected and 1.10^6 cells replated on human fibronectin-coated cells. The medium was exchanged every third day and the number of colonies finally assessed after 7 days.

Statistical methods

The values are presented in X ± SD; calculation for significance was done by means of ANOVA.

Results

In presence of risk factors CEC are increased and CEPC decreased (Tab. 2). Cigarette smoking (CEC) and hyperlipoproteinemia (CEPC) induce the most significant change, hypertension the least. Radioiodine therapy induces a significant change. The increase in CEC ranges from 34 to 103% (Tab. 3). The higher the dose, the more pronounced the peak and the later it occurs. The higher the therapeutic dose, the later the return to prevalues occurred. Recovery was faster for CEC as compared to CEPC, except after dosing with 200 mCi. CEPC showed a minimum at day 5 (5 and 20 mCi) and week 2 (80 and 200 mCi). The mean percent change ranged from –34 to –60% in a dose-dependent manner.

Discussion

Endothelial integrity is a major regulator of haemostatic balance. Although the relevant side effects of radioiodine therapy are well known, no data on the measures of endothelial function are available. Recently, a dose-dependent oxidation injury measuring the isoprostane 8-epi-$PGF_{2\alpha}$ in various compartments [4, 5] has

Table 3. Percent change (from baseline) in CEC and CEPC

interval	mCi	5		20		80		200	
n		17		11		6		8	
		CEC	CEPC	CEC	CEPC	CEC	CEPC	CEC	CEPC
day	1	10/4	2/3	12/4	–4/3	19/5	–3/3	17/5	–6/5
	2	25*/7	–15*/5	40*/4	–19*/8	46*/4	–26*/5	59*/6	–35*/9
	3	34*/6	–28*/7	56*/6	–31*/5	69*/6	–41*/6	86*/5	–47*/8
	5	30*/4	–34*/7	47*/8	–39*/6	65*/5	–43*/5	94*/8	–51*/14
week	1	16/5	–21*/8	36*/9	–36*/4	53*/5	–56*/12	103*/10	–60*/13
	2	4/3	–16/9	15/4	–29*/3	31*/7	–59*/10	86*/7	–60*/10
	3	1/3	–7/5	6/5	–17/5	18*/4	–26*/8	47*/9	–33*/6
	4	0/5	–3/4	4/3	–8/7	11/5	–23*/6	32*/11	–28*/7
	6	–2/4	4/5	1/7	–2/6	6/7	–16/9	7/10	–22*/8
	8	2/4	3/5	–2/6	–6/8	2/4	–7/5	11/6	–12/5
	10	3/6	5/6	2/3	–3/5	4/5	–4/7	5/5	–6/8
	12	1/6	1/5	3/4	2/4	0/4	–4/3	4/5	–1/7

data in mean values (% change); *) $p < 0.01$ (*vs.* prevalue); for abbreviations see Table 2

been described. We thus studied CEC and CEPC for functional characterization of endothelium. The CEC-count is inversely related to platelet survival [6] and a simple but reliable indicator of systemic haemostatic balance. The different methodological approaches to assess CEC [7, 8] all turned out to be quite reliable. The determination of CEC was originally introduced to assess (experimental) vascular injury [8]. Although the endothelial origin has been questioned earlier, morphological and (immuno-) chemical techniques clearly verified the nature of the cells [9]. An increased CEC-count has been found in cigarette smokers [10, 11], those with hyperlipidemia [12] and clinical manifestation of atherosclerosis [13, 14] or vascular disease [15–18] but not in those with hypertension. The simplicity of the technique easily facilitates follow-up monitoring. The findings after radioiodine therapy clearly show a temporary increase even at the lowest administered dose of 5 mCi. Although the prevalues severely depend on the respective risk factor profile of each patient, the kinetic behaviour is identical. The dose-dependent increase seems to achieve its maximum between 1 and 2 weeks after therapy. No significant interindividual day to day variations (unpublished data) make the parameter even more reliable. Together with the findings on lipoprotein modification, the data clearly indicate a significant temporary radiation injury at the local blood vessel wall level. Although the extent and duration makes clinical relevance as to vascular disease development and/or progression unlikely, the effect itself is of interest. It is of interest that with the risk factor groups CEC-prevalues in the high radioiodone dose-group are significantly higher. This points to the role of hypothyroidism as an additional factor increasing CEC. This claim has been verified by pilot investigations in patients featuring hypothyroidism and subsequently undergoing hormone substitution therapy.

CEPC are claimed to play a central role in the repair of endothelial damage. A decreased CEPC-count is associated with a decreased (re-)endothelialization capacity. Counting CEPC is an interesting new methodological approach in assessing endothelial functional behaviour [19, 20]. This role in vascular tissue repair after injury is well established.

Decreased CEPC are found in people at risk for the development of cardiovascular disease. A significant reduction in CEPC was reported [21] for hypercholesterolemia, diabetes and hypertension [22], confirming our findings (Table 2). Furthermore, a correlation in the testing of flow-mediated brachial artery reactivity and thereby endothelial (dys-)function has been described. Statin therapy [23], for example has been shown not only to mobilize and augment the number of CEPC but also to enhance their functional activity.

A number of reports also performed functional classification of CEPC, which has not been done, however, in this study.

Conclusion

Our findings indicate a significant haemostatic imbalance reflected by a disturbance in the endothelial lining due to both increased desquamation and diminished repair. Whether and to what an extent this may favour development and/or progression of vascular disease still remains to be assessed.

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