Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 59; Numer/Number 6/2008
ISSN 0423–104X

Serum concentration of hepatocyte growth factor (HGF) in oral squamous cell carcinoma before and after surgery. Preliminary report

Stężenia hepatocytowego czynnika wzrostu (HGF) we krwi chorych z rakiem płaskonabłonkowym błony śluzowej jamy ustnej przed i po leczeniu chirurgicznym. Doniesienie wstępne

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Abstract

Introduction: Generally, squamous cell carcinoma carries a poor prognosis because of its tendency to local invasion and subsequent metastasis, which is mediated by multiple factors and angiogenesis. Hepatocyte growth factor (HGF) is a potent mitogen for epithelial cells and regulates cell proliferation and migration and survival tumour angiogenesis and invasiveness.
The aim of the study presented here was to determine the serum concentration of HGF in patients with oral squamous cell carcinoma before and after initial treatment.
Material and methods: The investigation was carried out in a group of 10 men and 10 women who had been hospitalised at the Department of Craniomaxillofacial Surgery because of oral squamous cell carcinoma. Blood samples were collected before and four weeks after initial treatment. Concentrations of HGF were determined in all blood serum samples.
Results: In patients with oral squamous cell carcinoma daily oscillations of the mean values of HGF were significantly higher than those in healthy volunteers ($p < 0.001$). Serum concentrations of HGF were significantly higher in those patients who had undergone tumour and regional lymph node resection. Serum HGF levels correlated positively with primary tumour stage**.** In our study no significant association was observed between HGF levels and histological differentiation, but the daily oscillation of HGF was higher in those with G3 than in those with G2 and G1 status.
Conclusions: Our data showed that the changes can be important pathogenic elements involved in the progression of oral squamous cell carcinoma and may be a useful marker for clinical monitoring. **(Pol J Endocrinol 2008; 59 (6): 467–470)**

Key words: *oral squamous cell carcinoma, angiogenesis, HGF, TNM stage, histological grade*

Streszczenie

Wstęp: Rak płaskonabłonkowy błony śluzowej jamy ustnej charakteryzuje się wysokim stopniem inwazji miejscowej i wysoką częstotliwością przerzutów do szyjnych węzłów chłonnych. Proces inwazji i tworzenia przerzutu składa się z wielu połączonych wzajemnych relacji gospodarza i guza nowotworowego. Hepatocytowy czynnik wzrostu (HGF) jest potencjalnym mitogenem dla komórek nabłonkowych, który reguluje proliferację, ich ruchliwość, przeżycie, angiogenezę guza i inwazyjność.
Celem pracy była ocena stężenia HGF u chorych z rakiem płaskonabłonkowym jamy ustnej przed i po leczeniu chirurgicznym.
Materiał i metody: Badania przeprowadzono u 20 pacjentów, 10 mężczyzn i 10 kobiet. Grupę kontrolną stanowiło 20 zdrowych ochotników. Badania przeprowadzono przed oraz 4 tygodnie po zabiegu chirurgicznym.
Wyniki: Dobowe stężenia HGF przed leczeniem były znamiennie wyższe u chorych z rakiem płaskonabłonkowym jamy ustnej niż w grupie kontrolnej ($p < 0{,}001$). Stężenie HGF we krwi istotnie zwiększyło się u chorych po resekcji guza i regionalnych węzłów chłonnych. Obserwowano dodatnią korelację między stężeniem HGF a wielkością guza. Nie stwierdzono istotnego związku między stężeniem HGF a stopniem jego histologicznego zróżnicowania, nie mniej jednak obserwowano wyższe jego stężenia w przypadku raków ocenionych jako G3, niż w G2 i G1.
Wnioski: Wyniki badań sugerują, że HGF może odgrywać rolę w patogenezie oraz mieć znaczenie w monitorowaniu przebiegu klinicznego raka płaskonabłonkowego błony śluzowej jamy ustnej. **(Endokrynol Pol 2008; 59 (6): 467–470)**

Słowa kluczowe: *rak płaskonabłonkowy błony śluzowej jamy ustnej, angiogeneza, HGF, TNM, skala histologicznego zróżnicowania guza*

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Introduction

Oral cancer is a world health problem accounting for some 400 000 new cases annually [1]. Squamous cell carcinomas are the most frequent head and neck neoplasms and the sixth most frequent cancer in the world [2, 3]. Clinical and pathological parameters are inadequate for prognostic characterisation, since patients with equivalent tumour sites, TNM stages and histological grades may differ widely in the course of their disease and in survival. Treatment modalities include surgery, radiotherapy and chemotherapy, but disease control is limited, not least because approximately 25% of cases develop second primaries after first intervention [4].

Generally, squamous cell carcinoma has a poor prognosis because of its tendency towards local invasion and subsequent metastasis, which is mediated by multiple factors and angiogenesis [5]. Thus the identification of factors related to tumour genesis may lead to the development of novel tools to asses the malignancy of oral squamous cell carcinoma. The role of polypeptide growth factors in the pathogenesis in neoplastic diseases is currently the subject of several investigations. The mitogenic, differentiative, antiapoptotic and angiogenic properties of these peptides influence the proliferation of many cell types, including normal and transformed cells. There is evidence that tumour growth is dependent on angiogenesis, the formation of new blood vessels from pre-existing endothelium [6, 7]. Angiogenesis is a multi-step process involved in the proliferation, migration and differentiation of endothelial cells and is controlled by a finely tuned balance of angiogenic inducers and inhibitors [8]. Tumour cells produce several biologically active angiogenic factors which are known to play a crucial role in tumour angiogenesis in many different tumour types [9–11]. Hepatocyte growth factor (HGF) is a potent mitogen for epithelial cells, which regulates cell proliferation, migration, survival tumour angiogenesis and invasiveness [12]. Originally HGF was purified from rat serum and characterised as a substance that stimulates the growth of hepatocytes [13]. Currently, it is known as a pleotropic cytokine that acts on epithelial cells in several organs [14]. Because HGF is involved in cell proliferation, blood vessel formation and invasion, it is supposed that it plays a role in carcinogenesis and metastasis [15]. Some investigations suggest that HGF plays an important role as a paracrine factor in the invasion and metastasis of oral squamous cell carcinoma and an elevated serum level of HGF can be a predictive marker for metastasis formation in these patients [16].

This constitutes a preliminary report on serum levels of HGF in patients with oral squamous cell carcinoma before and after initial treatment. The research is being continued and will be presented in the next article.

The aim of the study presented here was to determine the serum concentrations of HGF in patients with oral squamous cell carcinoma before and after initial treatment.

Material and methods

The investigation was carried out before and four weeks after surgery in a group of 10 men and 10 women aged between 50 and 68 years who had been hospitalised at the Department of Craniomaxillofacial Surgery because of oral squamous cell carcinoma. All patients were staged according to the TNM classification of tumours. They had not received any previous treatment. The control group consisted of 20 healthy volunteers. None of the patients was found to be suffering from any infection or fever during the examinations. All had a normal body mass index (BMI) of 19–24. Patients with chronic diseases of the endocrine system and those with complaints of the circulatory, respiratory and excretory systems were excluded form the study. None of the patients received any medication during the seven days prior to the study or during the study period itself. The patients were treated using tumour and regional lymph node resection. An intravenous cannula was inserted into the ulnar vein of each of the patients studied. Blood samples were collected before and four weeks after surgery at 8.00 a.m., 2.00 p.m., 8.00 p.m. and 2.00 a.m. The serum was frozen at –75°C until assayed. In all blood serum samples the concentrations of HGF were determined in the laboratory in the Department of Pathophysiology. To measure HGF levels in serum a commercially available ELISA kit was used (Quantikine Human HGF Immunoassay; R&D System Inc). The results obtained were subjected to statistical analysis. $P < 0.05$ was accepted as significant. Results in pg/ml were expressed as the mean ± SD. The difference between the mean values of the analysed parameters was assessed using the Mann-Whitney U test or Student's *t*-test for unpaired variables. Homogeneity of variance was checked with the Fischer test.

Results

In patients with oral squamous cell carcinoma daily oscillations of the mean values of HGF were significantly higher than those in healthy volunteers both before and after the initial treatment ($p < 0.001$) (Fig. 1). However, serum concentrations of HGF were significantly higher in those patients who had undergone tumour and regional lymph node resection. Serum HGF levels correlated positively with primary tumour stage

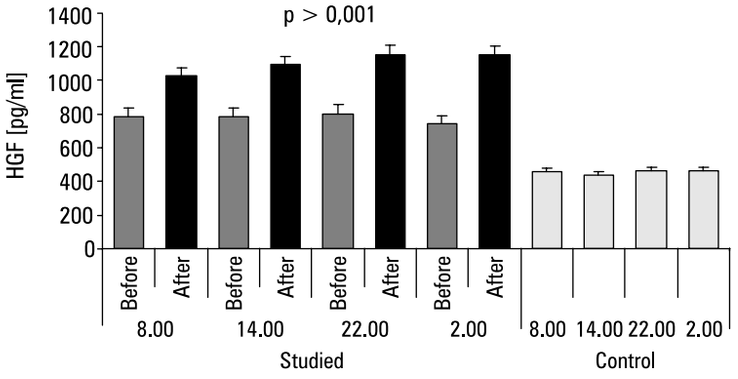

Figure 1. *Daily oscillations of the mean values of HGF [pg/ml] in the peripheral blood in patients with oral squamous cell carcinoma before and after the treatment as well as in the control group*

Rycina 1. *Dobowe wahania średnich wartości HGF [pg/ml] we krwi obwodowej u chorych na raka płaskonabłonkowego jamy ustnej przed i po leczeniu oraz u osób z grupy kontrolnej*

(Fig. 2). In our study no significant association was observed between HGF levels and histological differentiation, but the daily oscillation of HGF was higher in those with G3 than in those with G2 and G1 status (Fig. 3).

Discussion

Oral squamous cell carcinoma is characterised by a high degree of local invasion and a high rate of metastases to the cervical lymph nodes. Furthermore, these tumours show local recurrence after initial treatment, probably because of a tendency to micro-invasion and micrometastasis of the tumour cells at the primary site. Tumour growth and invasion involve multiple interactions not only by tumour cells but also between tumour and stromal cells and extracellular stroma. The process of metastasis and invasion consists of interactions between cancer and host cells. Several investigators have re-

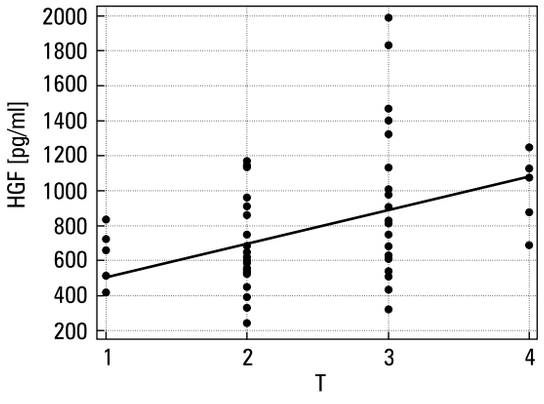

Figure 2. *Correlations between daily oscillations of the mean values of HGF in the peripheral blood and tumour stage (T) in patients with oral squamous cell carcinoma*

Rycina 2. *Korelacje między dziennymi wahaniami średnich wartości HGF we krwi obwodowej i stopniem zaawansowania guza (T) u chorych na płaskonabłonkowego raka jamy ustnej*

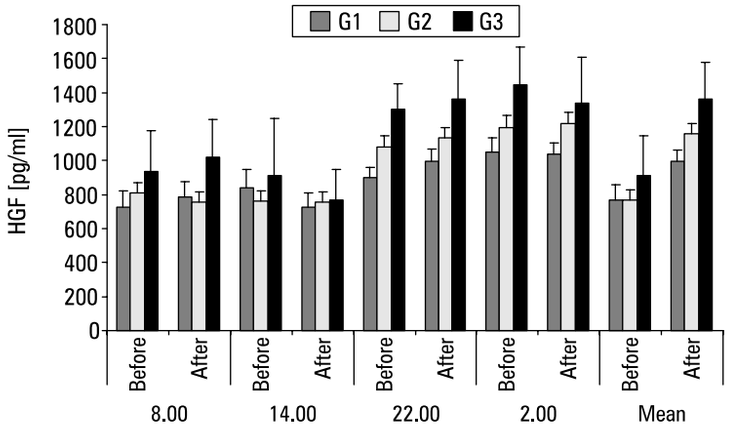

Figure 3. *Relationships between daily oscillations of the mean values of the HGF in the peripheral blood and histological differentiation of the tumour (G) in patients with oral squamous cell carcinoma*

Rycina 3. *Zależności między dobowymi wahaniami średnich wartości HGF we krwi obwodowej i histologicznym zróżnicowaniem guza (G) u chorych na płaskonabłonkowego raka jamy ustnej*

ported that local interactions between cancer cells and stromal cells, such as fibroblasts, lymphocytes or endothelial cells, are important for regulating angiogenesis, migration and invasion of tumour cells [17, 18]. HGF is mainly produced by mesenchymal cells such as fibroblasts. In fibroblast-derived factors, such as HGF, TGF-β, IL-1, IL-6, VEGF, b-FGF and TNF-α, hepatocyte growth factor is the most likely candidate for affecting the invasion and metastases of oral cancer [19]. Matsumoto et al. [20] reported that gingival fibroblasts enhanced the invasion of oral squamous cell carcinoma *in vitro*. In several studies a relationship between serum concentrations of HGF in cancer tissues and disease progression has been noted in patients with gastric, breast and lung cancer [21–23]. This relationship has not been reported in patients with oral squamous cell carcinoma. Uchida et al. [24] reported that elevated HGF levels in cancer tissue can be a predictive marker for metastasis formation in patients with oral squamous cell carcinoma. They also examined serum levels of HGF in these patients and showed significantly higher levels of HGF than in healthy volunteers. Furthermore, they showed that in patients who had residual tumours after surgery the serum levels of HGF did not significantly differ before and after treatment. No significant correlations were reported between serum levels of HGF and tumour size, lymph node metastases or the prognosis of the patients. In our investigation high serum levels of HGF were detected in patients with oral squamous cell carcinoma before treatment. After initial treatment a marked boost in serum HGF levels was observed. It is known that growth factors at supraoptimal concentrations sometimes inhibit the growth of cells [25–27]. On monolayer culture exogenous HGF did not stimulate cell growth but inhibited the growth at higher concentrations [24]. Thus it is likely that increased daily serum activity of HGF in patients after initial treatment of oral squamous cell carcinoma may inhibit cancer progression. We showed positive correlations between HGF serum levels and tumour stages. On the basis of these results, a higher preoperative serum level of HGF is shown to be closely related to a more advanced TNM stage. The preoperative level of HGF may thus reflect the severity of invasive oral squamous cell carcinoma. Chen et al. [16] also showed a significant association between HGF and tumour size. They reported that HGF concentrations were higher in T3 and T4 than in T1 and T2 oral squamous cell carcinoma. In our study no significant association was observed between HGF levels and histological differentiation, but the daily oscillation of HGF was higher in patients of G3 than those of G2 and G1 status. It is generally known that a higher G status corresponds to a poorer prognosis in oral squamous cell carcinoma.

Conclusions

Our data showed that changes in HGF serum concentration can be important pathogenic elements involved in the progression of oral squamous cell carcinoma and may be a useful marker for clinical monitoring.

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