Research Article

Lung level of HMBG1 is elevated in response to advanced glycation end product-enriched food *in vivo*

Babett Bartling[1], Christine Fuchs[1], Veronika Somoza[2], Bernd Niemann[1], Rolf-Edgar Silber[1] and Andreas Simm[1]

[1] Cardio-thoracic Surgery, Martin Luther University Halle-Wittenberg, Halle/Saale, Germany
[2] German Research Centre for Food Chemistry, Garching, Germany

High mobility group box protein 1 (HMGB1) is a ubiquitous nuclear protein that can be actively released from the cell in certain conditions thereby mediating cytokine-like function. While nuclear HMGB1 modulates the transcriptional activity of cells, extracellular HMGB1 partially acts *via* binding to the receptor for advanced glycation end products (RAGE), which is highly expressed in lung tissue. Therefore, we studied the impact of food-derived advanced glycation end products (AGEs), the Maillard reaction products, on the lung expression of HMGB1. Feeding rats with AGE-rich diet, containing either bread crust or coffee beverage, resulted in an upregulation of HMGB1 mRNA and protein especially in those animals receiving bread crust diet. The expression of RAGE was not influenced. Moreover, we revealed a positive correlation between an increased lung AGE level and HMGB1 protein expression in both animal groups receiving either bread crust or coffee extract but not in the control group. In contrast, the ageing-related AGE accumulation was not associated with an increased level of HMGB1 protein in lung tissue from senescent (100 wk) compared to young-adult (24 wk) rats. Our data suggest a physiological role of food- but not ageing-associated AGEs in the regulation of the HMGB1 expression in lung.

Keywords: Advanced glycation end products / HMGB1 / Lung / RAGE / Rat model

Received: November 2, 2006; revised: December 7, 2006; accepted: December 11, 2006

1 Introduction

The high mobility group box protein 1 (HMGB1) was originally described as a nonhistone DNA-binding protein [1] that serves as a structural compound critical for the proper transcriptional regulation in somatic cells [2]. HMGB1 is evolutionary conserved in eukaryotes and can be detected in almost all the cell and tissue types. However, its expression level and nuclear localization can vary with the type of tissue [3], development [4] and age [5]. The biological activity of HMGB1 is modulated by post-translational modifications, including acetylation, ADP ribosylation and methylation [6]. In the nucleus, HMGB1 binds to the minor groove of DNA modulating the transcriptional activity of steroid hormone receptors, p53, nuclear factor κB, homeobox-containing proteins and others [2]. The nuclear functions of HMGB1 are essential for the survival as HMGB1-deficient mice are born with a number of abnormalities and die within few hours due to hypoglycaemia [7]. Although the nuclear localization of HMBG1 seems to be the physiological basis of the HMBG1 function, HMGB1 is partially distributed in the cytoplasm [8] and can actively be released into the extracellular milieu by nonclassical secretion pathways. This has been predominately demonstrated for monocytes and macrophages, which secrete HMGB1 in response to proinflammatory cytokines or LPS stimulation [6], but several other types of cells are also able to secrete HMGB1 actively [4, 9]. In addition to the tightly controlled active secretion, HMGB1 can be passively released from the cell upon necrotic cell death thereby triggering systemic inflammation [10].

Secreted HMGB1 molecules mediate their extracellular biological function *via* binding to various interaction part-

Correspondence: Dr. Babett Bartling, Klinik für Herz- und Thoraxchirurgie, Martin-Luther-Universität Halle Wittenberg, Ernst-Grube-Str. 40, 06120 Halle/Saale, Germany
E-mail: babett.bartling@medizin.uni-halle.de
Fax: +49-345-557-7070

Abbreviations: AGEs, advanced glycation end products; **CML,** *N*-ε-carboxymethyllysine; **HMGB1,** high mobility group box protein 1; **MG,** methlyglyoxal; **RAGE,** receptor for advanced glycation end products

ners such as plasminogen, syndecan-1 and certain cell surface receptors. One of the HMGB1-binding receptor is RAGE, which has been originally isolated as a receptor for advanced glycation end products (AGEs) [11]. HMBG1 binds to RAGE in a dose-dependent and saturable manner that can be blocked by the administration of AGEs [12]. As RAGE has been extensively studied in the field of diabetic and inflammatory complications, various factors activated by RAGE are either proinflammatory cytokines or vascular adhesion molecules [13]. In contrast, the interaction of RAGE with HMGB1 induces extension of filopodia and neurite outgrowth [14] and controls the motility of neuronal and epithelial cells [15]. Altogether, these data suggest RAGE as a surface receptor mediating both pathophysiological and nonpathophysiological effects depending on the specific conditions and organs. This assumption is furthermore supported by the fact that lung tissue generally expresses RAGE at a very high level without associated organ dysfunction [11].

Although RAGE acts as a multiligand receptor interacting with several molecules in addition to AGEs and HMGB1 (*i.e.* S100 proteins, amyloids [13]), extracellular HMBG1 is believed to be the primary binding partner of RAGE in lung [12]. However, less is known about alterations of the HMGB1 expression in lung tissue in general and, in particular, in response to AGEs. Therefore, we studied the impact of AGEs on the expression level of HMBG1 in lung epithelial cells *in vitro* and in rat lung tissue *in vivo*. In this study, we used food-derived AGE analogues, the Maillard reaction products, which have been extracted from bread crust and roasted coffee beans. In addition to several animal feeding experiments, we analysed lung specimens from aged animals as the ageing process is physiologically associated with an increased accumulation of the tissue AGEs [16, 17].

2 Materials and methods

2.1 Animal models

Young-adult male Wistar rats (15 wk; Harlan-Winkelmann; Borchen, Germany) were used for the animal feeding experiments according to the protocol described before [18, 19]. Shortly, the animals were individually housed in metabolic cages under standard conditions and fed daily with an average of 30 ± 0.1 g of the diet *per* capita. The feeding experiments comprised three randomized groups (n = 6 rats *per* group) receiving a protein-free Altromin standard chow (C1004; Altromin, Lage, Germany), which was supplemented with 20% weight percentage of casein (control group). Of this control chow, 4.5% weight percentage of casein was replaced by either bread crust or lyophilized decaffeinated coffee beverage. Total fraction of defatted brown bread crust was used, which had been prepared from rye/wheat-mixed bread according to a protocol described earlier [20].

Table 1. Data from the rat models studied

	Age (wk)	Number	Body weight	Lung weight	% Lung weight
Treatment groups					
Control	15	6	347 ± 27	1.76 ± 0.49	0.51 ± 0.17
Bread crust	15	6	356 ± 24	1.81 ± 0.37	0.51 ± 0.11
Coffee extract	15	6	352 ± 17	2.07 ± 0.49	0.59 ± 0.15
Age groups					
Young-adult	24	9	483 ± 43	2.63 ± 1.34	0.55 ± 0.30
Senescent	100	9	494 ± 65	2.97 ± 0.20	0.60 ± 0.09

Data represent means ± SD.

Coffee beverage resulted from the powder of roasted coffee beans (*Coffee arabica*, Colombia, decaffeinated), which was percolated with hot water in a commercial coffee machine equipped with a cellulose filter (Melitta; Minden, Germany). Thereafter, the coffee beverage was defatted and freeze-dried as described before [18]. After the feeding period of 15 days, all the animals were fasted for 24 h and sacrificed by decapitation. Lung was removed, weighed, washed in ice-cold 0.9% NaCl and stored at −80°C until use. Moreover, lung tissue specimens obtained from male young-adult (24 wk) and senescent (100 wk) Spraque-Dawley rats (Harlan-Winkelmann) were used for analysing age-related differences. Detailed animal characteristics are summarized in Table 1. The local Animal Care and Use Committee approved the experimental procedures.

2.2 Cell lines, culture conditions and treatment

The human lung epithelial cell line H358 (ATCC cell bank; Manassas, VA, USA) was studied *in vitro*. Moreover, we analysed H358 cells stably overexpressing full-length human RAGE, which has been described earlier [21]. H358 were cultured in DMEM supplemented with 10% foetal calf serum (FCS), 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen; Karlsruhe, Germany) in a 10% CO_2 atmosphere at 37°C. A stock solution of solubilized bread crust (100 mg/mL) or coffee beverage (20 mg/mL) was prepared by adding either total bread crust or coffee beverage to DMEM medium. After 1 h incubation at room temperature on an orbital shaker, the stock solutions were cleared by centrifugation and the resulting supernatant was stored at −20°C for further use. Confluent H358 cells were treated after synchronization (withdrawal of FCS for 48 h) by adding different concentrations of solubilized bread crust (2 to 25 mg/mL) or extract from decaffeinated coffee (0.2 to 1 mg/mL) at a given time.

2.3 Protein extraction and immunoblot analysis

Protein from treated cells was prepared after washing the cells with PBS in SDS lysis buffer (10 mM Tris-HCl, 2% SDS, protease inhibitor mix (Sigma; Deisenhofen, Ger-

many); pH 7.4). Similarly, total protein from lung tissues was extracted after mechanical pulverization in liquid nitrogen and subsequent homogenization in SDS lysis buffer. Released nuclear DNA was removed by treating the tissue lysate with 25 U/mg Benzonase® (Merck; Darmstadt, Germany) in a Sonorex ultrasonic bath (Bandelin; Berlin, Germany) for 30 min at 37°C. Protein concentration was measured by the BCA protein assay (Pierce; Rockford, IL, USA), and samples containing 50 μg of total protein were mixed with 4 × loading buffer (500 mM Tris-HCl, 40% glycerol, 8% SDS, 80 mM DTT, 4 mM Na-EDTA; 0.1% bromophenol blue; pH 7.4), boiled for 2 min and loaded onto a SDS polyacrylamide gel. Proteins were electroblotted onto nitrocellulose membranes (Schleicher & Schüll; Dassel, Germany), blocked with 6% nonfat dry milk in TBS-T buffer (50 mM Tris-HCl, 300 mM NaCl, 0.15% Tween 20; pH 7.5) at room temperature for 1 h and incubated with the respective primary antibodies. Rabbit polyclonal antibodies against HMGB1 (BD Pharmingen; San Diego, CA, USA), RAGE [21]) and GAPDH (Abcam; Cambridge, UK) were applied in TBS-T buffer. The primary antibodies were detected by horseradish peroxidase-conjugated secondary antibodies (Dianova; Hamburg, Germany) and subsequently visualized by luminescence after incubation with one volume luminol substrate solution (2.5 mM luminol, 0.36 mM cumaric acid (Sigma)) and one volume 5.4 mM hydrogen peroxide in 100 mM Tris-HCl; pH 8.5. All the visualized signals were densitometrically estimated by use of the LAS 3000 computer-based imaging system (FUJIFilm; Tokyo, Japan) equipped with AIDA 3.5 software (Raytest; Straubenhardt, Germany).

For tissue AGE detection, 20 μg of the protein lysate was mixed with PBS and spotted onto nitrocellulose membranes using the Minifold Spot Blot Unit (Schleicher & Schüll). Comparable amount of each dotted protein sample was controlled by amido black staining of the membrane. A dilution series of methlyglyoxal (MG)-modified protein (in the case of argpyrimidine detection) or glucose-modified BSA (in the case of *N*-ε-carboxymethyllysine (CML) and unspecified AGEs detection) [22] was additionally loaded for creating a standard curve. After blocking the membrane with 6% nonfat dry milk in TBS-T buffer, tissue AGE levels were analysed by the use of the sheep polyclonal AGE-antibody (R. Schinzel; Vasopharm, Wuerzburg, Germany), and by the use of antibodies directed against defined AGE structures *i.e.* rabbit polyclonal anti-CML (E. Schleicher, University of Tuebingen, Germany) or mouse monoclonal anti-MG-arginine adducts (mAb6B; K. Uchida, University of Nagoya, Japan). The antibody mAb6B was directed to MG-modified protein and found to specifically recognize argpyrimidine [23]. Horseradish peroxidase-conjugated secondary antibodies and subsequent luminescence detected the primary antibodies as described above. The intensity of visualized signals was estimated by densitometry. A standard curve was created by graphically plotting the OD of the standard-specific signal *versus* the respective standard dilution. The highest standard dilution was determined as 1 arbitrary unit (a. U.) and the tissue AGE level was assessed by calculating the OD determined for the lung specimens corresponding to the standard curve.

2.4 HMGB1 immunocytochemistry

H358 cells grown on class coverslips were treated by adding different concentrations of solubilized bread crust or extract from decaffeinated coffee for 30 min. Thereafter, cells were washed with PBS, fixed in phosphate-buffered 4% formaldehyde for 10 min at 4°C. After membrane permeabilization with 1% Nonidet NP-40, cells were blocked with 10% BSA and stained for 1 h with the primary rabbit HMGB1 antibody (BD Pharmingen; 1:100 in PBS) at room temperature. HMGB1 detection was performed with the Alexa Fluor 594 antimouse IgG secondary antibody (Molecular Probes Europe; Leiden, Netherlands) and fluorescence microscopy equipped with a Spot RT camera (Zeiss; Jena, Germany). Omission of the primary antibody served as a negative control. Slides were embedded in Mowiol mounting medium (Merck; Bad Soden, Germany).

2.5 RNA preparation and expression analysis

Total RNA was isolated from lung tissues after mechanical pulverization in liquid nitrogen according to the protocol of Chirgwin *et al.* [24]. The RNA concentration was calculated from the absorption at 260 nm using the ND-1000 spectrophotometer (NanoDrop; Wilmington, DE, USA). The cDNA was synthesized from 500 ng of total RNA *per* 25 μL mixture with random hexamer primers and Superscript II™ reverse transcriptase (Invitrogen; Karlsruhe, Germany). 1 μL of the first-strand cDNA reaction was used as template for the PCR containing 2 × PCR mix (Promega; Mannheim, Germany) and 5 pmol of each gene-specific primer (rat HMBG1: 5′ CCC GGA TGC TTC TGT CAA CTT C 3′ (sense) and 5′ CCT CTT CAT CCT CCT CGT CGT C 3′ (antisense); rat RAGE: 5′ CCT GAG ACG GGA CTC TTC ACG CT 3′ (sense) and 5′ CAG AGC CCA CAT GAT CCA TGC T 3′ (antisense)) in a final volume of 25 μL. Equal quantity of synthesized cDNA was determined by the amplification of 18S rRNA (5′ GTT GGT GGA GCG ATT TGT CTG G 3′ (sense) and 5′ AGG GCA GGG ACT TAA TCA ACG C 3′ (antisense)). PCR was performed after initial denaturation at 95°C for 2 min, 30 s at 95°C, 20 s at primer annealing and 30 s at 72°C in a Mastercycler® (Eppendorf; Hamburg, Germany). After PCR amplification and gel-electrophoretic separation, the intensity of all the PCR products was evaluated using the LAS 3000 computer-based imaging system (FUJIFilm) and AIDA 3.5 software (Raytest). The mRNA expression values were normalized as ratio *per* 18S rRNA.

2.6 Statistics

The significance of comparison of mean values was determined by the Student's *t*-test, and the one-way ANOVA procedure was used for multiple comparisons in the case of normally distributed data (SigmaStat software; Jandel Corp., San Rafael, CA, USA). Data reported are means ± SD with $P < 0.1$ as indicating a tendency and $P < 0.05$ as indicating a significant difference of mean data. The significance of the correlation coefficient R for linear regression analyses was tested using the *two-sided* test.

3 Results and discussion

3.1 Effect of AGE-rich food compounds on the HMGB1 level in lung epithelial cells

Bread crust and coffee extract were used for studying the impact of food-related AGE analogues on the protein expression of HMGB1 in human H358 lung epithelial cells. The short-term treatments of H358 cells with either bread crust or coffee extract commonly downregulated HMGB1 after 30 min of incubation, which was partially recovered after 120 min (Fig. 1A). As H358 cells express RAGE at a very low level compared with normal lung tissue [21], we studied H358 cells stably overexpressing RAGE but did not find an influence on the downregulation of HMGB1 in these conditions (data not shown). Subsequent long-term experiments also revealed a diminished amount of HMGB1 in response to both AGE-rich foods dependent on the time of incubation and the concentration applied (Fig. 1B). Solely coffee extract slightly induced the HMGB1 protein expression at a concentration of 1 mg/mL (Fig. 1B). The altered protein level of HMGB1 might result from directly controlled transcription machinery in response to AGE-enriched food. Human *hmbg1* has a strong TATA-less promoter, whose activity can immediately be reduced to one-sixth due to the stimulation of a silencer element upstream

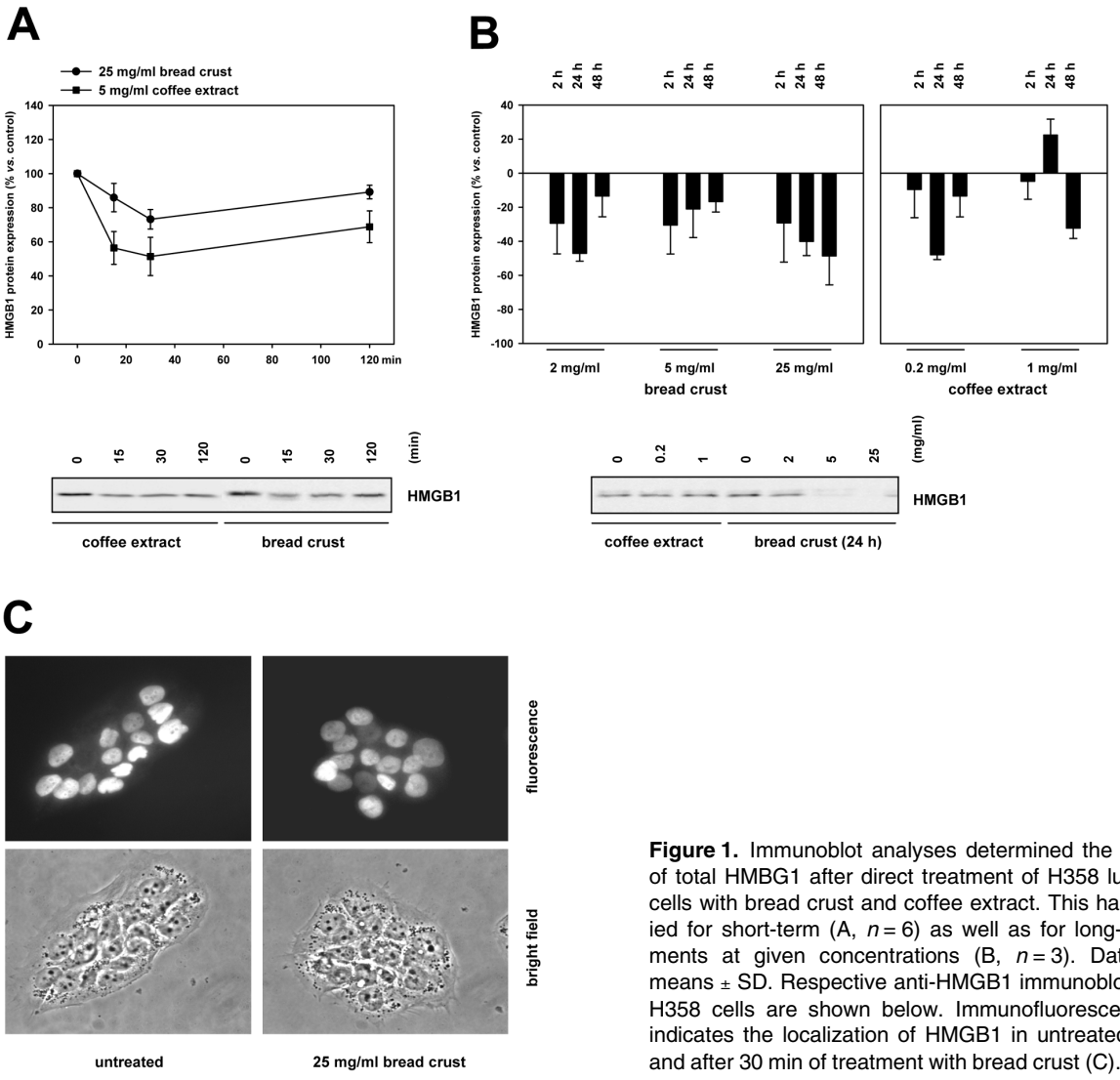

Figure 1. Immunoblot analyses determined the protein level of total HMBG1 after direct treatment of H358 lung epithelial cells with bread crust and coffee extract. This has been studied for short-term (A, $n = 6$) as well as for long-term experiments at given concentrations (B, $n = 3$). Data represent means ± SD. Respective anti-HMGB1 immunoblots of treated H358 cells are shown below. Immunofluorescence staining indicates the localization of HMGB1 in untreated H358 cells and after 30 min of treatment with bread crust (C).

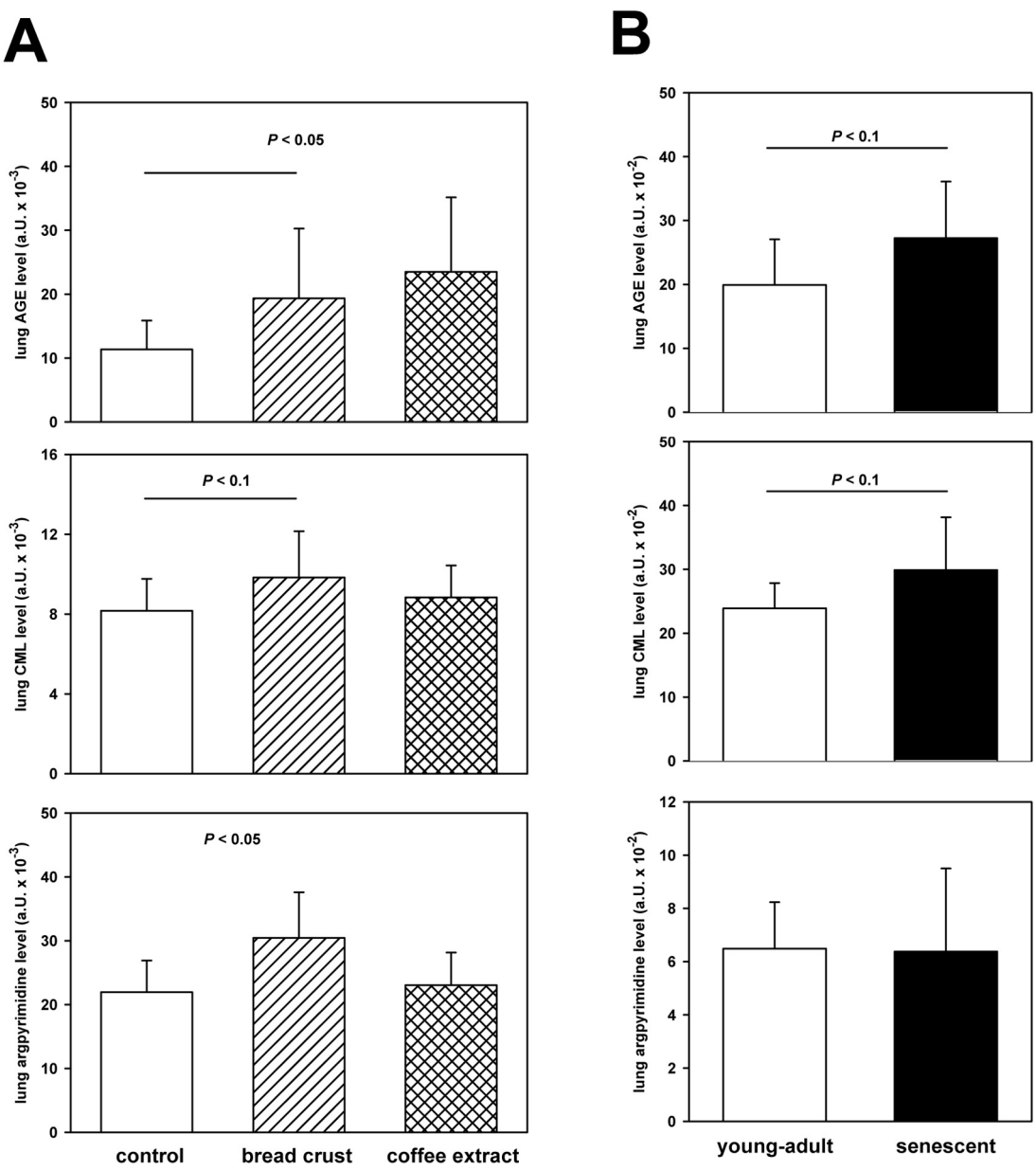

Figure 2. Spot blot analyses of lung tissue with subsequent antibody detection determined the level of unspecified AGEs and the defined AGE compounds CML and argpyrimidine in rats after feeding them with food enriched with bread crust or coffee extract compared to control rats (A; $n = 6$ each diet group). Moreover, the age-associated accumulation of those AGEs was quantified in lung tissue derived from young-adult and senescent rats (B; $n = 9$ each age group). All the data are given as mean ± SD.

of the promoter [25]. However, the protein level of HMGB1 might also be diminished by a sudden release of HMGB1 from the cell. Therefore, H358 cells were additionally analysed by immunocytochemistry detecting the primary localization of HMGB1 in the nucleus with marginal HMGB1 staining in the cytoplasm (Fig. 1C). The direct treatment of H358 cells with bread crust (Fig. 1C) or coffee extract (not shown) did not induce the relocalization of HMGB1 that suggests the downregulation of HMGB1 at the level of expression. Furthermore, the unaffected nuclear staining of HMGB1 points at its main function in the nucleus of these lung epithelial cells.

Although the downregulation of HMGB1 in response to bread crust and coffee extract indicated a possible biological role of those AGE compounds in the lung epithelium, it has to emphasize that the epithelial cells studied are transformed cells. Transformed cells generally express HMGB1 at a high level as demonstrated for a number of tumour specimens and tumour cell lines [2]. Consequently, the impact of AGE analogues might be different as lung epithelial cells

express HMGB1 at physiological extent. Moreover, food-derived AGEs are modified by digestive mechanisms *in vivo* [26] thereby changing their physiological potential. For that reason, we subsequently analysed lung tissue from rats that received an AGE-rich diet, which was supplemented with either bread crust or coffee extract.

3.2 Correlation of lung AGE level and HMGB1 expression after AGE-rich diet

Feeding rats with AGE-enriched foods resulted in an increased level of AGEs in lung tissue after 2 wk of feeding (Fig. 2A). Although it is uncertain whether the food-derived AGEs directly accumulate in the lung tissue or induce secondary mechanisms, the amount of the unspecified AGEs was higher in both feeding groups, bread crust and coffee extract group. The defined AGE structures CML and argpyrimidine were only elevated in the animal group receiving bread crust diet (Fig. 2A). In contrast to the higher tissue AGE levels mediated by AGE-enriched foods, the ageing-associated increase in the unspecified AGEs was less pronounced in lung (Fig. 2B). Deposition of the AGE structure CML tended to be increased in senescent rats compared to young-adult rats, too, whereas argpyriminde was not altered in the aged lung tissue. On the basis of ageing as a physiological process associated with the accumulation of endogenously formed AGEs in inner organs, the increased amount of lung AGE structures in aged rats was relatively low. However, this might firstly base on the fact that we analysed AGE modifications of total protein preparations and not of extracts enriched with long-lived proteins. In this regard, it has been shown that particularly long-lived matrix proteins including collagens are modified by AGEs in aged lung [17]. Moreover, we determined the tissue AGE levels by immunodetection methods, which do not record all the potential AGE structures.

Subsequent molecular analyses of the lung tissue revealed an increased mRNA expression of HMGB1 in response to the AGE-rich foods (Fig. 3A). This has been demonstrated for both animal feeding groups either receiving bread crust or coffee extract diet. Although an elevated level of translated HMGB1 protein had only resulted from the bread crust-enriched food, we determined a positive correlation between the amount of the unspecified AGEs and HMGB1 protein in rat lung tissue of both feeding groups (Fig. 3B). In contrast, the expression of the HMGB1- and AGE-binding receptor RAGE was not affected by feeding animals with AGE-enriched food (Figs. 3C and D).

Our data highly suggest a potential role of food-derived AGE analogues in the regulation of the HMGB1 expression in inner organs including lung. Although the biological outcome of this elevated HMGB1 expression remains still elusive in these conditions, overexpression studies revealed HMGB1 as a cell-protective protein [27, 28] as long HMGB1 is not released from the cell in pathophysiological conditions [10]. This protective potential is associated with the transcriptional downregulation of the proapoptotic protein Bak and induction of the antiapoptotic protein Bcl-x_L [27, 28], thereby mediating cellular resistance against chemotherapy-induced cell death [27, 28]. However, when applying both AGE compounds directly on the lung epithelial H358 cells, we rather induced an impaired cell proliferation associated with cell death induction at higher concentrations [29]. These differential results between the cell-protective and cell-damaging potential of food-derived AGEs might also explain the contrary protein expression of HMGB1 *in vitro* and *in vivo* (Figs. 1A and B and Figs. 3A and B).

On the basis of a similar relation between the AGE amount and the HMGB1 expression of mRNA and protein, respectively, in the animal feeding group receiving bread crust diet (Fig. 3A), *hmgb1* seems to be modified at the stage of transcription. These results support data showing that AGEs are able to stimulate the gene expression by modulating the levels of promoter-binding proteins as recently described for collagen IV and α-smooth muscle actin in mesangial cells [30]. The transcription of HMGB1 is controlled by general transcription factors such as Sp1 and AP-1. In particular, activation of the AP1 complex might be an important AGE-related target that controls the gene activity of *hmgb1* as AP1 has been shown to be activated in response to AGE structures *in vitro* [22]. However, the direct relation between an increased amount of unspecified AGEs and HMGB1 expression at mRNA and protein, respectively, was less pronounced in those rats receiving coffee extract (Fig. 3A). These data suggest the potential of distinct food-derived AGE structures, which are only able to induce the expression of HMGB1. Moreover, other biological mechanisms could secondarily affect the expression level and stability of HMGB1 *in vivo* as well as the cytoplasmic localization and subsequent release of HMGB1 in these conditions.

3.3 Lung HMGB1 expression does not correlate with ageing-associated AGEs

Previous publications revealed a higher protein expression of HMGB1 in rat lung tissue in response to ageing [5] and to the age-/AGE-related Alzheimer's disease [31]. Therefore, we were interested whether the AGE accumulation related to age [16] does correlate with the tissue level of HMGB1 in lung. Like feeding animals with AGE-rich food, ageing resulted in a higher mRNA expression of HMGB1 in senescent compared to young-adult rats (Fig. 4). This increase was very small at mRNA level and could not be detected at protein level anymore (Fig. 4) contrasting to early findings of Prasad and Thakur [5], who found an elevated HMGB1 protein level in senescent rats. However, Prasad and Thakur used another biochemical approach for HMGB1 protein quantification.

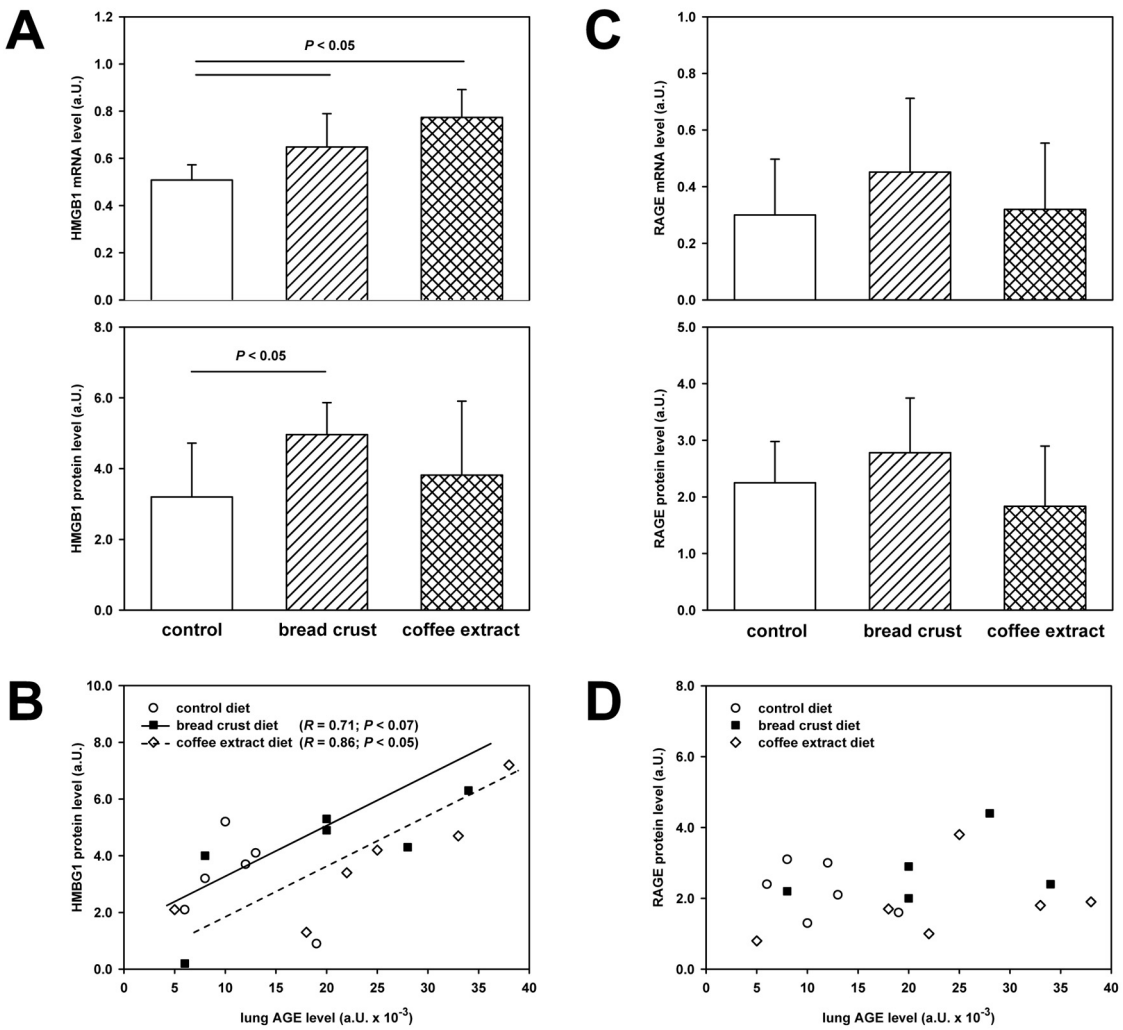

Figure 3. The impact of AGE-enriched food, containing either bread crust or coffee extract, on the lung level of HMGB1 (A) and the surface receptor RAGE (C) was studied for the protein and mRNA expression compared to control rats ($n = 6$ each group). Additionally, we analysed direct correlations between the protein expression of HMGB1 (B) or RAGE (D) and the AGE level (detection of undefined AGE compounds) in rat lung tissue for each diet group. Data represent means ± SD.

In contrast to the animal feeding groups receiving an AGE-rich diet, we did not find any direct correlation between the endogenous AGE level determined in ageing lung specimens and the corresponding HMGB1 protein expression (data not shown). The protein level of the receptor RAGE was also not influenced by ageing. The unaltered expression of HMGB1 in aged lung might be partially explained by a less induced increase in tissue AGEs as compared to the animal feeding groups (Figs. 2A and B). However, other age-related factors are possible as well. Firstly, tissue AGE structures accumulating with age might be different from those accumulating with an AGE-enriched diet. Secondly, molecular mechanism related to the biological ageing process might conversely affect the expression level of the HMGB1 protein. Furthermore, a number of posttranslational modifications including acetylation and glycosylation are potential mechanisms modifying not only the stability but also biological activity of HMGB1 [6], which cannot be assessed by the expression analyses performed in our study.

4 Concluding remarks

HMGB1 is a nonhistone DNA-binding factor that primarily modulates the transcriptional activity in the nucleus of cells. By studying lung tissue, we demonstrated that the expression of this essential protein is elevated in response to an AGE-rich nutrition but not in response to the ageing-associated AGE modifications *in vivo*. These findings propose the potential role of food-derived AGE compounds in modifying the transcription of eukaryotic cells.

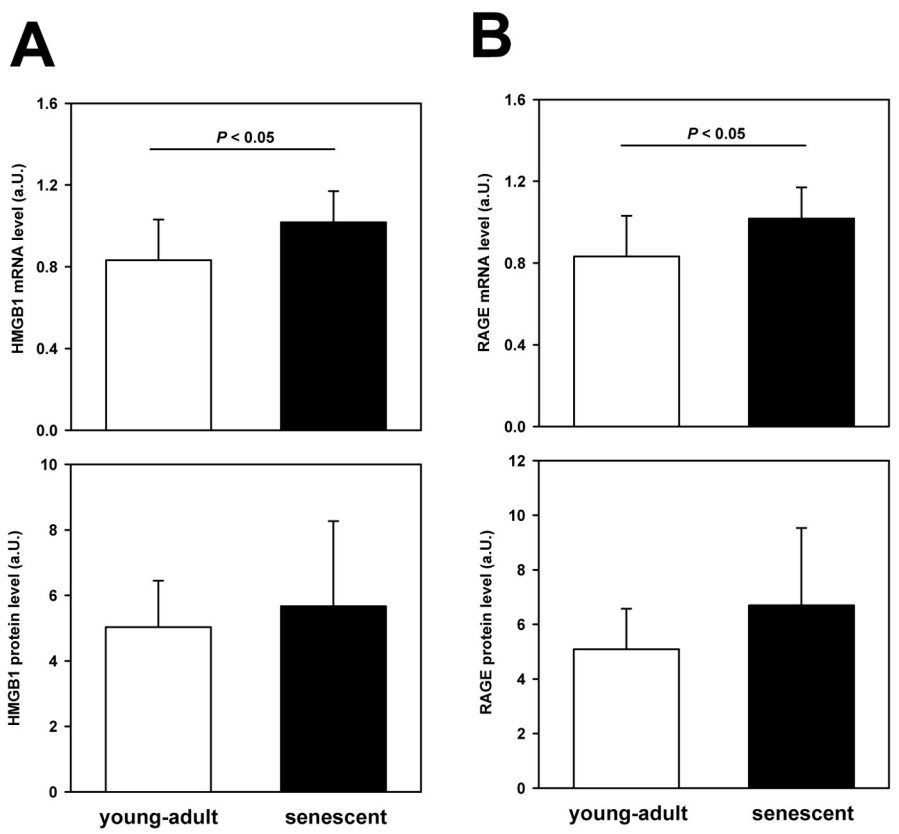

Figure 4. Age-dependent analyses of rat lung tissue studied the protein and mRNA expression level of HMGB1 (A) and RAGE (B) in senescent compared to young-adult rats ($n = 9$ each age group). Data are means ± SD.

The authors thank Sabine Koitzsch for technical assistance and Professor Dr. Thomas Hofmann for providing the bread crust and coffee extracts. This project was supported by the Wilhelm Roux grant of the Bundesministerium für Bildung und Forschung, BMBF (FKZ7/04; FKZ13/01), by the Deutsche Forschungsgemeinschaft DFG (Si-1317/1-1) and by the FEI (Forschungskreis der Ernaehrungsindustrie e.V., Bonn, Germany), the AiF and the Ministry of Economics and Technology (Project No. AIF-FV 12403 N).

5 References

[1] Goodwin, G. H., Sanders, C., Johns, E. W., A new group of chromatin-associated proteins with a high content of acidic and basic amino acids, *Eur. J. Biochem.* 1973, *38*, 14 – 19.

[2] Muller, S., Ronfani, L., Bianchi, M. E., Regulated expression and subcellular localization of HMGB1, a chromatin protein with a cytokine function, *J. Intern. Med.* 2004, *255*, 332 – 343.

[3] Mosevitsky, M. I., Novitskaya, V. A., Iogannsen, M. G., Zabezhinsky, M. A., Tissue specificity of nucleo-cytoplasmic distribution of HMG1 and HMG2 proteins and their probable functions, *Eur. J. Biochem.* 1989, *185*, 303 – 310.

[4] Rauvala, H., Pihlaskari, R., Isolation and some characteristics of an adhesive factor of brain that enhances neurite outgrowth in central neurons, *J. Biol. Chem.* 1987, *262*, 16625 – 16635.

[5] Prasad, S., Thakur, M. K., Distribution of high mobility group proteins in different tissues of rats during aging, *Biochem. Int.* 1990, *20*, 687 – 695.

[6] Ulloa, L., Messmer, D., High-mobility group box 1 (HMGB1) protein: friend and foe, *Cytokine Growth Factor Rev.* 2006, *17*, 189 – 201.

[7] Calogero, S., Grassi, F., Aguzzi, A., Voigtlander, T., *et al.*, The lack of chromosomal protein Hmg1 does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice, *Nat. Genet.* 1999, *22*, 276 – 280.

[8] Isackson, P. J., Bidney, D. L., Reeck, G. R., Neihart, N. K., Bustin, M., High mobility group chromosomal proteins isolated from muclei and cytosol of cultured hepatoma cells are similar, *Biochemistry* 1980, *19*, 4466 – 4471.

[9] Fages, C., Nolo, R., Huttunen, H. J., Eskelinen, E., Rauvala, H., Regulation of cell migration by amphoterin, *J. Cell. Sci.* 2000, *113*, 611 – 620.

[10] Scaffidi, P., Misteli, T., Bianchi, M., Release of chromatin protein HMGB1 by necrotic cells triggers inflammation, *Nature* 2002, *418*, 191 – 195.

[11] Neeper, M., Schmidt, A., Brett, J., Yan, S., *et al.*, Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins, *J. Biol. Chem.* 1992, *267*, 14998 – 15004.

[12] Hori, O., Brett, J., Slattery, T., Cao, R., *et al.*, The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin. Mediation of neurite outgrowth and co-expression of rage and amphoterin in the developing nervous system, *J. Biol. Chem.* 1995, *270*, 25752 – 25761.

[13] Bucciarelli, L. G., Wendt, T., Rong, L., Lalla, E., *et al.*, RAGE is a multiligand receptor of the immunoglobulin superfamily: implications for homeostasis and chronic disease, *Cell. Mol. Life Sci.* 2002, *59*, 1117 – 1128.

[14] Huttunen, H. J., Fages, C., Rauvala, H., Receptor for advanced glycation end products (RAGE)-mediated neurite outgrowth and activation of NF-kappaB require the cytoplasmic domain of the receptor but different downstream signaling pathways, *J. Biol. Chem.* 1999, *274*, 19919 – 19924.

[15] Huttunen, H. J., Fages, C., Kuja-Panula, J., Ridley, A. J., Rauvala, H., Receptor for advanced glycation end products-binding COOH-terminal motif of amphoterin inhibits invasive migration and metastasis, *Cancer Res.* 2002, *62*, 4805 – 4811.

[16] Wolff, S., Jiang, Z., Hunt, J., Protein glycation and oxidative stress in diabetes mellitus and ageing, *Free Radic. Biol. Med.* 1991, *10*, 339 – 352.

[17] Bellmunt, M. J., Portero, M., Pamplona, R., Muntaner, M., Prat, J., Age-related fluorescence in rat lung collagen, *Lung* 1995, *173*, 177 – 185.

[18] Somoza, V., Lindenmeier, M., Wenzel, E., Frank, O., *et al.*, Activity-guided identification of a chemopreventive compound in coffee beverage using *in vitro* and *in vivo* techniques, *J. Agric. Food Chem.* 2003, *51*, 6861 – 6869.

[19] Somoza, V., Wenzel, E., Lindenmeier, M., Grothe, D., *et al.*, Influence of feeding malt, bread crust, and a pronylated protein on the activity of chemopreventive enzymes and antioxidative defense parameters *in vivo*, *J. Agric. Food. Chem.* 2005, *53*, 8176 – 8182.

[20] Lindenmeier, M., Faist, V., Hofmann, T., Structural and functional characterization of pronyl-lysine, a novel protein modification in bread crust melanoidins showing *in vitro* antioxidative and phase I/II enzyme modulating activity, *J. Agric. Food Chem.* 2002, *50*, 6997 – 7006.

[21] Bartling, B., Hofmann, H., Weigle, B., Silber, R., Simm, A., Down-regulation of the receptor for advanced glycation endproducts (RAGE) supports nonsmall cell lung carcinoma, *Carcinogenesis* 2005, *26*, 293 – 301.

[22] Simm, A., Munch, G., Seif, F., Schenk, O., *et al.*, Advanced glycation endproducts stimulate the MAP-kinase pathway in tubulus cell line LLC-PK1, *FEBS Lett.* 1997, *410*, 481 – 484.

[23] Oya, T., Hattori, N., Mizuno, Y., Miyata, S., *et al.*, Methylglyoxal modification of protein. Chemical and immunochemical characterization of methylglyoxal-arginine adducts, *J. Biol. Chem.* 1999, *274*, 18492 – 18502.

[24] Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., Rutter, W., Isolation of biologically active ribonucleic acid isolated from sources enriched in ribonuclease, *Biochemistry* 1979, *18*, 5294 – 5299.

[25] Lum, H. K., Lee, K. L., The human HMGB1 promoter is modulated by a silencer and an enhancer-containing intron, *Biochim. Biophys. Acta.* 2001, *1520*, 79 – 84.

[26] Finot, P., Magnenat, E., Metabolic transit of early and advanced Maillard products, *Prog. Food Nutr. Sci.* 1981, *5*, 193 – 207.

[27] Nagatani, G., Nomoto, M., Takano, H., Ise, T., *et al.*, Transcriptional activation of the human HMG1 gene in cisplatin-resistant human cancer cells, *Cancer Res.* 2001, *61*, 1592 – 1597.

[28] Brezniceanu, M. L., Volp, K., Bosser, S., Solbach, C., *et al.*, HMGB1 inhibits cell death in yeast and mammalian cells and is abundantly expressed in human breast carcinoma, *FASEB J.* 2003, *17*, 1295 – 1297.

[29] Bartling, B., Rehbein, G., Somoza, V., Silber, R. E., Simm, A., Maillard reaction product-rich food impair cell proliferation and induce cell death *in vitro*, *Signal Transduct.* 2005, *6*, 303 – 313.

[30] Iehara, N., Takeoka, H., Yamada, Y., Kita, T., Doi, T., Advanced glycation end products modulate transcriptional regulation in mesangial cells, *Kidney Int.* 1996, *50*, 1166 – 1172.

[31] Takata, K., Kitamura, Y., Kakimura, J., Shibagaki, K., *et al.*, Role of high mobility group protein-1 (HMG1) in amyloid-beta homeostasis, *Biochem. Biophys. Res. Commun.* 2003, *301*, 699 – 703.