POINT OF VIEW

ANALYTICAL CHEMICAL CONSIDERATIONS ON TUMOR GENESIS

L. Campanella
Chemistry Department, University of Rome "La Sapienza", Roma, Italy

НЕКОТОРЫЕ ПРИЧИНЫ ВОЗНИКНОВЕНИЯ ОПУХОЛЕЙ: ТОЧКА ЗРЕНИЯ ХИМИКА–АНАЛИТИКА

Л. Кампанелла
Римский университет "Ла Сапиенца", Рим, Италия

The differences in the content of both heavy metals and free radicals in tumors and normal tissue of kidneys have been analyzed. Concentration of free radicals was measured by means of a superoxide dismutase biosensor that has been recently developed in our laboratory. Two main differences were observed: 1) higher metal concentration in normal tissues than in tumor ones; 2) higher concentrations of radicals and toxic agents, as particularly benzene, in tumor tissue with accompanying lower concentrations of radical scavengers. Such differences in radical concentrations can be related to: lowered superoxide dismutase activity in tumor tissue and production of free radicals by means of so named secondary metabolism pathways of some compounds adsorbed from the environment.
Key Words: biosensor, superoxide dismutase, heavy metals, free radicals.

Определяли концентрации тяжелых металлов и свободных радикалов в опухолевой и непораженной тканях почки человека. Концентрацию свободных радикалов в ткани измеряли с помощью оригинального, созданного автором супероксиддисмутазного биосенсора. Обнаружено, что: 1) в нормальной ткани почки определяется более высокая концентрация некоторых металлов (медь, кадмий, цинк), чем в опухолевой; 2) в опухолевой ткани почки выявлено более высокое содержание свободных радикалов и ряда токсических веществ, в частности бензола, чем в непораженной, при более низкой концентрации таких поглотителей свободных радикалов, как цистеин. Пониженное содержание свободных радикалов в опухолевой ткани может быть обусловлено снижением активности супероксиддисмутазы и продукцией свободных радикалов в реакциях так называемого вторичного метаболизма ряда веществ, которые поступают в ткани организма из окружающей среды.
Ключевые слова: биосенсор, супероксиддисмутаза, тяжелые металлы, свободные радикалы.

The analytical determinations show an increasing concentrations of some common pollutants (heavy metals, hydrocarbons, especially benzene, pesticides, namely organophosphoric and carbammic ones) in the environmental compartments (water, air, soil). The increasing concentrations of organic pollutants in the environment can result in their progressive accumulation in target organs of human body with formation of DNA adducts and following cellular degeneration. The organism defends itself with metabolic activities finalised to consume these accumulated species. The unique new method using SOD biosensor has been employed to monitor free radical as well as to determine heavy metal and benzene concentrations in both normal and tumor tissues.

The following samples of kidney tissue have been analyzed in the study: 31 samples of embryonic kidney, 12 samples of the kidneys of newborns, 37 samples of non–cancer patients and 68 samples of 34 cancer patients (both normal and cancer tissue). The weight of the samples ranged from 0.3 to 3.0 g. All samples for the determination of the concentration of heavy metals have been prepared in a conventional analytical way after repeated mineralisation. Normal kidney tissue samples have been compared with tumor ones. Metal concentrations were determined by means of "inductively coupled plasma'' method and atomic absorption spectrometry. Benzene analysis was performed with gas chromatography and mass spectrometry.

The concentrations of free radicals in tumor and normal tissues were measured by means of SOD biosensor that has been recently developed in our department [1–5]. It is based on the use of SOD (EC 1.15.1.1) physically entrapped in a Kappa–carrageenan gel membrane and is supplemented with an amperometric sensor for hydrogen peroxide. The enzymatic membrane is sandwiched between a dialysis membrane and a cellulose acetate membrane (the latter in contact with H_2O_2 electrode), ensuring the selectivity of the electrode by preventing the passage of several electroactive substances that could cause interference, being oxidized at the platinum anode, which is polarized at + 0.7 V.

SOD catalyses the dismutation reaction of the superoxide radical with the formation of O_2 and H_2O_2 according to the following reaction:

$$O\cdot_2^- + O\cdot_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2.$$

The state of the enzymatic reaction was then monitored by the amperometric sensor for H_2O_2. The alternative way based on monitoring O_2 was abandoned as the calibration of the sensor was performed by producing known amounts of the superoxide radicals and

Received: December 13, 1999.
*Correspondence. E–mail: luigi.campanella@uniroma1.it
Abbreviation used: SOD — superoxide dismutase.

this production is based on the system xantine/xantine oxidase involving oxygen too.

The hydrogen peroxide produced is oxidized by the working electrode according to the following reaction:

$H_2O_2 \xrightarrow{\text{Pt wire (0.7 V)}} O_2 + 2H^+ + 2e^-$,

thus generating a signal of diffusion limit current proportional to the concentration of $O\cdot_2^-$ radical in solution.

Italian National Health Institute recommendations were adopted for all the experimental procedures.

Minimum and maximum concentrations of several heavy metals being analyzed in the samples under study were the following: Cu: 0.5–13 ppm; Cd: 0.01–40 ppm; Zn: 3–60 ppm. In the majority of the cases studied the concentration of these metals in normal kidney tissue was higher than in tumors (see Table).

Table. Metal concentrations in normal (C_N) and tumor(C_T) kidney tissue:

Metal	Data of the comparisons	Number of samples	Ratio range (r) between C_N and C_T
Cu	$C_N > C_T$	46	1.5–10
	$C_T > C_N$	4	
Cd	$C_N > C_T$	50	4–3000
	$C_T > C_N$	0	
Zn	$C_N > C_T$	45	1.8–9
	$C_T > C_N$	5	

At the same time the contents of radicals and toxic agents, benzene in particular, in tumor samples being analyzed were higher than in normal ones with accompanying lower concentrations of radical scavengers, especially cystein, in tumor tissues. In the experimental setting upon the addition of the known concentrations of radicals *in situ* to the normal and tumor samples of the same weight a rapid consumption of the radicals was registered in normal samples while in tumor tissue such a consumption was much lower and slower**.**

The difference in radical concentrations between tumor and normal kidney tissues can be related to the lowered SOD activity in tumor tissue as well as production of free radicals by means of the so named secondary metabolism pathways of some compounds absorbed from the environment. For instance, dioxygenase is able to catalyze the metabolism of benzene to trans, trans–muconic bringing to the secondary metabolism.

Benzene in its secondary metabolism can initiate generation of free radicals being able to attack DNA. Moreover, the secondary catabolic pathway of benzene could be the cause of the metabolic deviation in the reaction which transforms piruvic acid into Ac–CoA. As the result of such deviation piruvic acid goes to lactic acid. Consequently lactate dehydrogenase is diverted from its function of regenerating NAD from NADH in order to reactivate glucose catabolism.

In the case of benzene the metabolic capacity of kidney tumor and healthy tissues due to dioxygenase activity was measured *in vitro*. Higher values in the tumor in comparison with healthy tissues were recorded suggesting activation of defense mechanisms. Unfortunately the metabolic reaction produces also such toxic metabolites as muconaldehyde. On the other side the high concentrations of radicals can be related also to their consumption from environment and foods as well as to the deficiency of radical scavengers, first of all metallothioneines, particularly cystine and cysteine, both being very reactive with heavy metals. They are also accumulated in human organs, according to a Lewis acid–base reaction, with subsequent sequestrations. The acid constants of cysteine are:

$pK_{carb} = 1.71$; $pK_{amm} = 10.77$; $pK_{subs} = 8.36$, where:

pKcarb is the pK of the carboxylic group dissociation;

pKamm is the pK of the protonated ammonium group;

pKsubs is the pK of the successive dissociation after the first two of ammonium and carboxylic group.

pK values of the complex between cysteine and some common metals range between 7 and 20, so corresponding to the stable compounds.

The recovery mechanism must so provide to the deficiency of radicals scavengers by supplying non toxic substances such as cysteine and derivatives, vitamin C, and other antioxidants having these functions and finalized to consume the excess of the present radicals. The specific single enzymatic equipment must also be evaluated; in some cases this can be defective or less active so furtherly weakening the defense mechanisms that only can be reactivated by giving the lacking enzymes.

Therefore, carcinogenic environmental pollutants are accumulated in tissues, particularly metals binding cysteine. Such elevated heavy metal concentrations in kidney tissue may be considered as one of the main reasons of defective detoxification function of kidneys with resulting accumulation of harmful metabolic substances including free radicals. At the same time increased radical concentration in tumors could be the consequence of the lowered SOD activity. Two strategies could be followed as to eliminate this danger. First, it is necessary to supplement the body with radical scavengers that are able to decrease radical concentrations and to overcome their inhibiting action on the enzymes. As a preventive measure it is also desirable to enrich the oxidative enzymatic reactions especially in the cases of their deficiency upon exposure to the factors increasing the risks of cancer.

REFERENCES

1. **Campanella L, Favero G, Tomassetti M.** A modified amperometric electrode for the determination of free radicals. Sensors Actuators 1997; **B 44**: 559–65.

2. **Campanella L, Favero G, Mastrofini D, Tomassetti M.** Further developments in toxicity cell biosensors. Sensors Actuators 1997; **B 44**: 279–85.

3. **Campanella L, Favero G, Tomassetti M.** A Biosensor for determination of free radicals. In: R Puers, ed. Proc Eurosensors X. 10th Europ Conf Solid-State Transducers. Leuven, 1996; **3**: 917–8.

4. **Campanella L, Favero G, Persi L, Tomassetti M.** A superoxide dismutase biosensor to evidence the anti free-radical properties of healthy and cancerous kidney tissues. Como: SIB-BIB, 1999.

5. **Campanella L, Favero G, Persi L, Tomassetti M.** New sensors and biosensors for superoxide radical to evidence molecules of biomedical and pharmaceutical interest having scavenger properties. In: 8th Intern Meet Recent Developments in Pharmaceutical Analysis. Roma, 1999.