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A note on the effect of feeding genetically modified potatoes on selected indices of non-specific resistance in rats

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ABSTRACT

An experiment was conducted on 16 male rats of initial body weight 150 g divided into 2 groups and fed during 5 weeks on balanced semi-synthetic diets containing non-modified or genetically modified potatoes (GM). The modification consisted of repression of the gene encoding ADP-ribosylation factor of protein and increased synthesis of 14-3-3 protein. Feeding GM potatoes increased the number of bacteria phagocytized by monocytes, percentage of neutrophils producing reactive oxygen species, and oxygen-dependent bactericidal activity of neutrophils.

KEY WORDS: GM potatoes, phagocytic activity, oxidative burst, rats

INTRODUCTION

Potatoes are commonly used in human nutrition. Changing the chemical composition of potato tubers using biotechnological methods would allow modification of the supply of specific amino acids, sugars, organic acids, flavonoids or

mineral components in the human diet. In animal feeding, potatoes with an altered chemical composition may improve animal performance and dietetic value of the products.

Biotechnological intervention into the genetic structure of plants may induce, however, dramatic changes, since together with the synthesis of expected compounds, other unspecified elements may appear. The immune system of animals and humans is one of the most sensitive systems that may respond to dietary compounds recognized by the organism as foreign.

The objective of the present study was to determine the effect of feeding genetically modified (GM) potatoes on selected indices of non-specific resistance in rats. Genetic modification of potatoes consisted of repressing the gene encoding ADP-ribosylation factor (ARF) of protein and intensification of the 14-3-3 protein synthesis (Wilczyński et al., 1997).

MATERIAL AND METHODS

Two semi-synthetic iso-protein diets containing potatoes (*Solanum tuberosum* L.cv. Desiree), non-modified (control diet) or subjected to genetic modification (GM, experimental diet), were used. The diets were composed of (%): casein, 12.0; cellulose, 3.2; soya oil, 4.0; mineral-vitamin mixture, 5.0; DL-methionine, 0.1; dried potatoes, 20.0, and maize starch, 55.7. Each diet was fed for 5 weeks to 8 male rats of an initial body weight of 150 g maintained in individual cages at room temperature (21°C), 60% humidity, 12/12 h dark/light. The health status of the animals was checked every day and body weight was recorded weekly. At the end of the experiment the animals were fasted for 12 h and anaesthetized by injection of ketamine. Blood was sampled from the heart into heparinized tubes, cooled to 4°C and analyzed. Phagocytic activity of neutrophils and monocytes of whole blood and oxidative burst activity of neutrophils were determined using Phagotest and Bursttest (Orpegen Pharma, D-69115 Heidelberg, Germany) by flow cytometry (Bohmer et al., 1992).

RESULTS AND DISCUSSION

The GM potato tubers derived from plants with augmented synthesis of proteins 14-3-3, modulators of enzyme activities. Protein 14-3-3 regulates nitrogen assimilation by binding to nitrate reductase, sucrose synthesis by binding to sucrose phosphate synthase, cell communication by direct interaction with plasma-membrane H+-ATPase, and the cell cycle by binding to cdc25 phosphatase and exporting it to the nucleus in response to DNA damage (Finnie et al., 1999).

The chemical composition of GM potatoes differed from that of non- GM tubers. They contained more starch and simple sugars, and less protein, ash and organic acids than non-GM potatoes (Wilczyński et al., 1998; Kosieradzka and Sawosz, unpublished). Feeding GM potatoes to rats did not affect their apparent health and growth but resulted in considerable differentiation of the non-specific immunological response, determined as the phagocytic and bactericidal activity of neutrophils and monocytes (Table 1). While the percentage of monocytes as well as of neutrophils phagocytizing opsonized *E. coli* bacteria did not differ

TABLE 1

Phagocytic activity of neutrophils and monocytes in peripheral blood of control and experimental rats

Cells	Parameters	Groups		SEM
		control normal potato	experimental GM potato	
Neutrophils	Percent of phagocytizing cells	78.75	78.50	2.25
	Mean fluorescence intensity (4 decades, 1025 channels, log)	591.25	657.75	56.81
Monocytes	Percent of phagocytizing cells	73.0	79.25	3.58
	Mean fluorescence intensity (4 decades, 1025 channels, log)	349.50	457.0*	25.32

* significant difference ($P<0.05$)

between groups, a significant increase of fluorescence intensity, corresponding to the number of bacteria phagocytized by monocytes was observed in rats from the experimental group. A similar, though insignificant, tendency was found in neutrophils. Monocytes belong to phagocytic cells occurring in the blood system in small numbers, they are usually present in tissues in the form of macrophages (2-4%).

Phagocytic activity, as indicated by the intensity of fluorescence of opsonized and fluorescein-labelled *E. coli* bacteria, depends on many factors, and among others, on sensitivity of the cell to chemotactic signals and components of complement, IgG immunoglobulins, capacity of the cellular membrane to form convections, cooperation of phagosomes and lysosomes, and also on the synthesis of bactericidal factors.

In the study, the increase of the percentage of neutrophil granulocytes producing reactive oxygen species (ROS), as affected by stimulation with opsonized *E. coli* bacteria and by the action of fMLP (N-formylo-methionylo-leucylo-phenyloalanine), a component of bacterial walls, was observed in GM-potato-fed rats

(Table 2). Moreover, a considerable increase of fluorescence intensity, corresponding to the oxygen-dependent, bactericidal activity of neutrophils, as affected by the stimulation with opsonized *E. coli*, was also found. The stimulation of neutrophil granulocytes with phorbol esters (PMA), both in relation to the percentage of phagocytizing cells and to fluorescence intensity, proceeded in the control group on a level similar to the stimulation of these cells by *E. coli*, whereas in the experimental group, the intensity of fluorescence was lowered. The oxidative burst consists of synthesis of the superoxide anion radical and, also, of hydrogen dioxide, and may result in further generation of free radicals with a higher toxicity-the hydroxyl radical or hypochloric ion (Harris, 1992), leading to degradation of cellular structures. Hirt et al. (1994) observed reduced phagocytosis in patients with sepsis, renal failure, recurrent bacterial infections, and reduced burst activity in patients with chronic granulomatous disease. Some data indicate that exposure to particulate pollution is likely to impair host defense functions of macrophages and monocytes (Becker and Soukup, 1998).

The observed increase of non-specific resistance due to feeding GM potatoes may result from the intake of a component of GM potatoes, inducing the immunological response of monocytes and neutrophils. Many chemical compounds can modify the oxidative burst of phagocytizing cells, including lectins, oligosaccharides, polyunsaturated fatty acids, and flavonoids (Middleton and Kandaswami, 1992; Hughes and Pinder, 1997; Stuart et al., 1997). Therefore, univocal interpre-

TABLE 2

Oxidative burst activity of neutrophils in peripheral blood of control and experimental rats

Stimulus	Parameters	Groups		SEM
		control normal potato	experimental GM potato	
E. coli	Percent of oxidizing cells	89.20	92.50*	1.28
	Mean fluorescence intensity (4 decades, 1025 channels, log)	14.81	42.80*	2.17
fMLP[1]	Percent of oxidizing cells	51.0	81.25*	6.24
	Mean fluorescence intensity (4 decades, 1025 channels, log)	3.46	4.59	0.49
PMA[2]	Percent of oxidizing cells	85.0	87.0	3.21
	Mean fluorescence intensity (4 decades, 1025 channels, log)	23.97	15.65	9.08

[1] fMLP – N-formylo-methionylo-leucylo-phenyloalanine

[2] PMA – phorbol 12-myristate 13-acetate

* significant difference ($P<0.05$)

tation of the results would require further, more profound analytical studies of the GM potatoes used in the experiment and also determination of the precise mechanism of inducing the phagocytic activity observed in the experiment.

CONCLUSIONS

Feeding GM potatoes (with the repression of the gene encoding ADP-ribosylation factor of protein and the increased synthesis of protein 14-3-3) to rats resulted in an increase of the number of bacteria phagocytized by monocytes, percentage of neutrophils producing reactive oxygen species, and oxygen-dependent bactericidal activity of neutrophils.

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STRESZCZENIE

Wpływ ziemniaków modyfikowanych genetycznie na wybrane wskaźniki odporności nieswoistej u szczurów

Doświadczenie przeprowadzono na 16 szczurach samcach o początkowej m.c. 150 g. Zwierzęta podzielono na dwie grupy i żywiono przez 5 tygodni pełnowartościowymi dietami izobiałkowymi zawierającymi ziemniaki niemodyfikowane lub modyfikowane genetycznie (GM). Modyfikacja polegała na represji genu kodującego czynniki ADP rybozylacji białka i zwiększeniu syntezy białka 14-3-3. Żywienie dietą zawierającą ziemniaki GM spowodowało zwiększenie liczby bakterii fagocytowanych przez monocyty, odsetek neutrofili syntetyzujących reaktywne formy tlenu oraz tlenozależnej aktywności bakteriobójczej neutrofili.