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Department of Biology and Genetics, Medical University of Lublin

JOLANTA RZYMOWSKA, JOANNA ŁUSZCZAK, MARTA ZIAJA-SOŁTYS

Antioxidant activity of superoxide dismutase and glutathione peroxidase in cattle tissue

Antyoksydacyjna aktywność dysmutazy ponadtlenkowej i peroksydazy glutationowej w tkankach bydła

INTRODUCTION

Bovine cysticercosis induced by *Taenia saginata* is a zoonotic disease warranting routine inspection measures for the postmortem detection of cysticerci (cysts), because infected cattle are a source of human infection if affected beef is eaten raw or undercooked [13, 15]. In the year 2006, 97, whereas in 2007, 84 intestinal cestode infections caused by this tapeworms were registered in Poland [11, 17, 18]. In Europe *Taenia saginata* is still present and incur economic loses due to condemnation, refrigeration and downgrading of infected slaughter cattle [7]. *Taenia saginata* inhabits the tissues of cattle during its larval stage and infects the intestine of humans in its adult form. *Cysticerci* have been shown to exist in the lymphatic vessels of the heart, skeletal muscle and liver [16].

The parasite infection causes activation of the host immune system components [14]. Acute or chronic tissue damage has been described in the cells of hosts infected with different species of parasites due to the increased amount of reactive oxygen radicals which cause lipid peroxidation [6].

Products of lipid peroxidation (free radicals) formed in various biochemical reactions are normally effectively scavenged by antioxidants that also suppress the actions of reactive oxygen substances. Antioxidant defences include both enzymatic and nonenzymatic systems. Superoxide dismutase, glutathione peroxidase and catalase are the principal enzymatic antioxidants. The enzymatic factors are reduced glutathione, vitamin C, vitamin E, β-carotene, ceruloplasmin and bilirubin, which may function as antioxidants [4,10].

There is, to our knowledge, no study which evaluates the antioxidants status in the Cysticercosis, therefore the aim of this paper is to assess the activities of SOD and GPx in the cattle tissues infected with *Taenia saginata*.

MATERIAL AND METHODS

In this study superoxide dismutase and glutathione peroxidase activity was investigated in the skeletal muscles taken from 50 cattle carcasses naturally infected with *Taenia saginata* and 6 healthy

heads of cattle (from Meat Factory „Łmeat" in Lukow, Poland). The tissue samples (1 gram each) were washed in phosphate buffered saline, homogenized in 50 mM Tris-HCl, 1 mM EDTA; pH 6,8 buffer at 4^0C and centrifuged. By using Randox kits (RANSEL - Glutathione Peroxidase and RANSOD - Superoxide Dismutase) the activities of SOD and GPx in the supernatants of muscle tissue homogenates (20mg/ml) were determined.

In 10% sodium dodecyl sulfate (SDS) - polyacrylamide gel electrophoresis the protein samples (50 µg) from lysates were separated and transferred to nitrocellulose membranes (Millipore). The membranes were blocked with 1% defatted milk in TBS buffer, pH 7.5 with 0.1% Triton X-100 and then exposed to commercially available primary antibodies (Glutathione peroxidase 1 antibody, Abcam; Cu/Zn SOD antibody, GeneTex). Then the membranes were incubated with secondary antibodies conjugated with alkaline phosphatase (Sigma) for two hours at room temperature.

By reaction of NBT (nitroblue tetrazolium) and BCIP (5-bromo-4-chloro-3-indolyl-phosphate) in buffer (100 mM Tris-HCl, pH 9,5, 100 mM NaCl) the bands were visualized. The band intensities were compared with the control ones.

RESULTS

Our results showed that the activity of the SOD and GPx measured spectrophotometrically was increased in skeletal muscle tissues of slaughter infected cattle. SOD activity was over two fold higher (SOD 15612.5 U/g; p=0.00003) (Fig. 1), when compared with the activity of this enzyme in the control group (SOD 7610.0 U/g), whereas glutathione peroxidase activity in tissues of the animals infected *Taenia saginata* was higher (1373.0 U/g; p=0.0008) in comparison with the studied group of healthy animals (549.8 U/g) (Fig. 2).

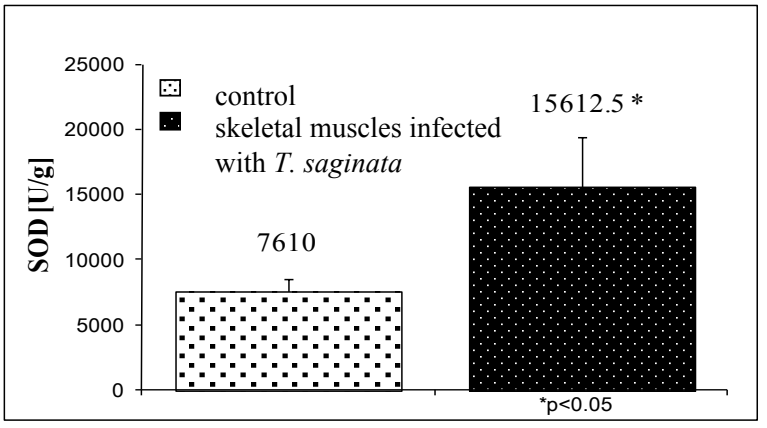

Fig. 1. Comparison of superoxide dismutase (SOD) activity in skeletal muscles of the control and *Taenia saginata* infected cattle tissues

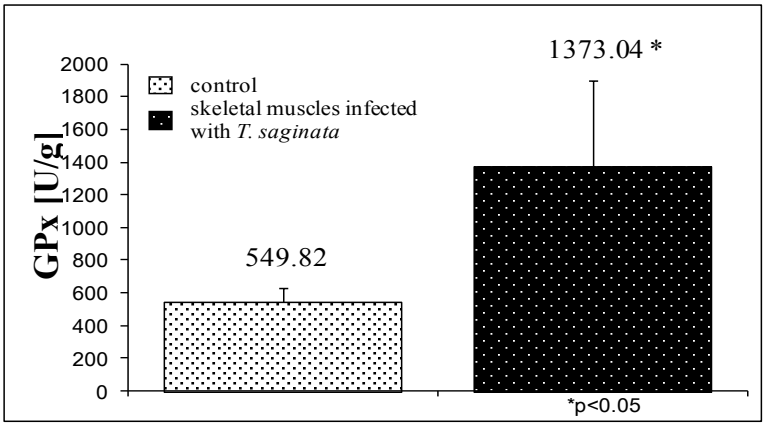

Fig. 2. Comparison of glutathione peroxidase (GPx) activity in skeletal muscles of the control and *Taenia saginata* infected cattle tissues

Western blot tissue extracts analysis detected a 16 kDa band for superoxide dismutase, which corresponded with the value of single monomer CuZnSOD, and its activity was compared with separate samples infected with tapeworms. SOD synthesis stimulation appeared in the host tissues containing cysticercus in contrast with the control samples (Fig. 3). There glutathione peroxidase as a protein band with the molecular mass amounting to 22 kDa was identified (Fig. 4). There were no significant differences in the glutathione peroxidase band intensities observed the in the studied samples in comparison with the control animals.

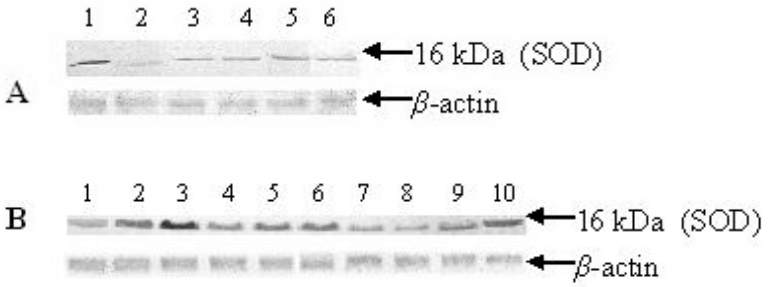

Fig. 3. Examples of immunoblot reaction patterns of representative skeletal muscles from cattle infected by *Taenia saginata* compared to the healthy animals. SOD protein was found to have a molecular size of 16 kDa. A. lane 1–6: controls; B. lane 1–10: infected muscle samples

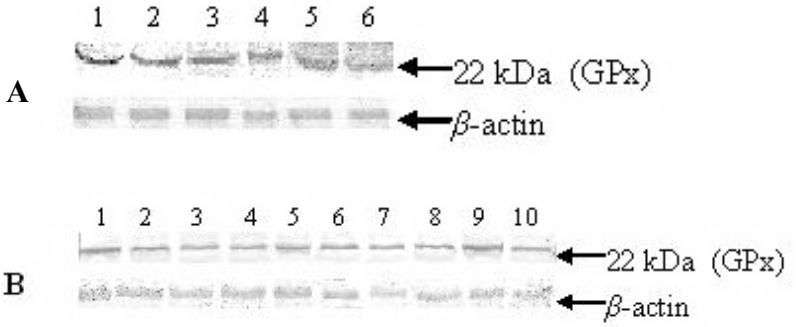

Fig. 4. Examples of the immunoblot reaction patterns of representative skeletal muscles from cattle infected by *Taenia saginata* compared to the healthy animals. GPx protein was found to have a molecular size of 22 kDa. A. lane 1–6: controls; B. lane 1–10: infected muscle samples

DISCUSSION

The first step during infection of the parasite is reaction of the host organism by activating complement-depending immune response [12]. Highly reactive oxygene species (O_2^{-}, H_2O_2 and $^{-}$OH) generated by activated phagocytes, including eosinophils and neutrophils, represent a powerful effector mechanism against parasites [1, 3, 8,14].

Superoxide dismutase and glutathione peroxidase belong to the main defense antioxidants that prevent the formation of new free radical species. They convert the existing free radicals into less harmful molecules before they have a chance to react. SOD converts superoxide into hydrogen peroxide and GPx changes hydrogen peroxide into harmless molecules. Thus they prevent superoxide and hydrogen peroxide from interacting and producing hydroxyl and singlet oxygen through Fe-dependent Fenton reaction [6].

In the present study, we observed a statistically significant increase (relative to the control value in uninfected cattle) in the activity of antioxidant SOD (spectrophotometrically measured and by western blot technique) and GPx enzymes (spectrophotometrically measured) in skeletal muscles infected by *Taenia saginata* (Fig. 1–3). There were observed no significant differences in the activity of glutathione peroxidase in the examined samples from cattle muscles and in control groups found using the western blot technique (Fig. 4).

Intensification of the antioxidant processes could be related to the presence of *Taenia saginata* larvae, which may induce phagocytes to generate free radicals [6]. These results are supported by other studies indicating that such antioxidant enzymes as SOD and GPx functioning as a defence mechanism against oxygene-derived free radicals may play an important role in the host's biochemical defence mechanisms during cysticercosis [2, 5, 6, 9 12].

The results presented here led us to conclude that the imbalance between pro- and anti-oxidant processes play a main role in the pathology associated with cysticercosis. The increase in activity

of the antioxidant enzymes in tissues of infected animals is a response to oxidizing stress which can be caused by the presence of the parasite in animals muscles. The changes observed in the activity of antioxidant enzymes in the tissues infected with tapeworm larvae can help to develop new and effective methods of detecting measles in slaughter animals.

CONCLUSIONS

Increase in the activity of antioxidant enzymes in tissues of infected animals is a response to oxidizing stress which can be caused by the presence of the parasite in animals muscles. These results support the significance of antioxidant processes in host defense mechanism during parasitic infections. Thus, it is stated that slaughter wastes animals with detected measles constitute a very important element of fighting with cysticecosis and taeniasis. Moreover, the changes observed in the activity of antioxidant enzymes in the tissues infected by tapeworm larvae can help to develop new and effective methods of detecting measles in slaughter animals.

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SUMMARY

In this study the activity of two antioxidant enzymes, superoxide dismutase (EC 1.15.1.1) and glutathione peroxidase (EC 1.11.1.9) was examined in skeletal muscles from cattle infected with *Taenia saginata*. We observed a statistically significant increase (relative to the control value in uninfected cattle) in the level of enzymes in spectrophotometric analysis (superoxide dismutase, glutathione peroxidase) and western blot technique (superoxide dismutase). There were no significant differences in the activity of GPx in cattle tissue infected with cysticercus in comparison with the control healthy animals assessed with western blot technique.

Our results suggest that SOD and GPx enzymes are important in the host's biochemical defence mechanisms during cysticercosis.

Key words: superoxide dismutase, glutathione peroxidase, *Taenia saginata*, antioxidant, cattle

STRESZCZENIE

Zbadano aktywność dysmutazy ponadtlenkowej (SOD) i peroksydazy glutationowej (GPx) w mięśniach szkieletowych bydła zarażonego *Taenia saginata.* Za pomocą analizy spektrofotometrycznej zaobserwowano statystycznie znaczący wzrost poziomu obu enzymów w porównaniu z kontrolą – zwierzętami zdrowymi. Oznaczenie poziomu antyoksydantów, przeprowadzone metodą *western blot*, wykazało istotnie wyższą aktywność dysmutazy ponadtlenkowej u bydła rzeźnego w stosunku do zwierząt niezarażonych *Taenia saginata.* Nie stwierdzono znaczących różnic w poziomie aktywności peroksydazy glutationowej. Wzrost aktywności dysmutazy ponadtlenkowej i peroksydazy glutationowej w tkankach zwierząt zarażonych jest odpowiedzią na stres oksydacyjny, wywołany obecnością pasożyta w mięśniach zwierząt. Poznanie zmian aktywności tych enzymów może być istotne w wykrywaniu wągrzycy zwierząt, a także jest niezmiernie ważnym elementem profilaktyki tasiemczycy u ludzi.

Słowa kluczowe: dysmutaza ponadtlenkowa, peroksydaza glutationowa, *Taenia saginata*, antyoksydant, bydło