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Some Lysosomal Enzyme Profiles in Children with Nephroblastoma: The Effect of Nephrectomy

[1]Donatien Gatsing, [2]H. Ibrahim Garba, [3]Roseline Aliyu,
[4]H. Abiodun Adebayo, [5]P. Obekpa and [3]I. Godwin Adoga
[1]Department of Biochemistry, Faculty of Science,
University of Dschang, P.O. Box 67, Dschang, Cameroon
[2]Industrial Chemistry Programme, School of Science,
Abubakar Tafawa Balewa University, P.M.B. 0248, Bauchi, Nigeria
[3]Department of Biochemistry, Faculty of Medical Sciences,
University of Jos, P.M.B. 2084, Jos, Nigeria
[4]College of Science and Technology, Covenant University,
P.M.B. 1023, Canaanland OTA, OGUN State, Nigeria
[5]Department of Surgery, Jos University Teaching Hospital (JUTH), Jos, Nigeria

Abstract: The serum levels of some lysosomal enzymes-namely β-glucuronidase (EC 3.2.1.31), β-galactosidase (EC 3.2.1.23) and acid phosphatase (EC 3.1.3.2)-were measured in controls, pre-operative and post-operative nephroblastoma patients. β-glucuronidase activity showed a significant ($p<0.05$) increase before surgical intervention, followed by significant ($p<0.05$) decreases 24 h, 72 h and 7 days after operation. The activities of β-galactosidase and acid phosphatase showed no significant ($p>0.05$) changes before and after surgical intervention. These data indicate that nephroblastoma and surgery may have a marked effect on the serum levels of some lysosomal enzymes.

Key words: Nephroblastoma, nephrectomy, β-glucuronidase, β-galactosidase, acid phosphatase

INTRODUCTION

Nephroblastoma is one of the most common malignant tumour of the kidney and presents in the first decade and often in the first year of life, most commonly as an abdominal mass (Davison and Lambie, 1991). It is an embryonic type of tumour derived from kidney rudiments and forms a large well-circumscribed growth which rapidly invades blood vessels (Govan *et al*., 1991). Nephroblastoma is the most common renal neoplasm in children, accounting for about 90% of paediatric kidney tumours and 6% of all childhood cancers (Anglesio *et al*., 2004).

Disease states usually lead to moderate or extensive tissue damage depending on the time of onset and severity of the disease (Garba *et al*., 2006). The major factor known to be responsible for changes in serum levels of intracellular enzymes is injury to organs or tissues rich in such enzymes (Garba *et al*., 2005a). Such conditions are usually associated with the release of enzymes from the diseased organ or tissue into circulation. The consequence is an increase in activity of such enzymes in body fluids (Garba *et al*., 2005b; Gatsing *et al*., 2005). Thus, measurement of enzymatic activity in serum/plasma and other body fluids has been employed in the diagnosis of diseases (Garba *et al*.,

Corresponding Author: Donatien Gatsing, Department of Biochemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon Tel: + 237 751 67 40

2006). Enzymatic methods have proven to be crucial to the diagnosis of acute myocardial infarction (Adams *et al*., 1994; Garba *et al*., 2005b), liver diseases (Castaldo *et al*., 1994) and acute pancreatitis (Patenghini and Pagani, 1989). Apart from their value as general diagnostic indicators, enzyme activity measurements in body fluids are providing scientists and clinicians with new insights into the pathological basis of some diseases (Garba *et al*., 2006). In some instances, they are used as predictors of the progress of diseases, particularly certain types of cancers (Gatsing *et al*., 2000, 2002, 2003; Garba *et al*., 2005a). The activity of lysosomal enzymes is increased in body fluids during inflammation, in which cellular malfunction and cellular death occur (Vlacha *et al*., 2004).

Although the value of the use of lysosomal enzymes excreted in urine as non-invasive tests for renal nephropathy has been documented by several authors (Kunin *et al*., 1978; Price, 1982; Adoga and Glew, 1995), little is known specifically about the serum activities of lysosomal enzymes in nephroblastoma.

In the present study, we measured the serum activities of three different lysosomal hydrolases-namely β-glucuronidase (EC 3.2.1.31), β-galactosidase (EC 3.2.1.23) and acid phosphatase (EC 3.1.3.2)-in pre-operative and post-operative patients suffering from nephroblastoma, with a view to assessing the effect of the disease and surgery (nephrectomy) on these enzyme levels, that might prove valuable as non-invasive means of monitoring this pathological condition.

MATERIALS AND METHODS

Blood Specimens and Serum Preparation

Blood samples for enzyme analyses were obtained from 3- to 7- year-old pre-operative and post-operative male patients, in the paediatrics wards of the Jos University Teaching Hospital (JUTH), suffering from nephroblastoma (n = 11). All of these patients had cancer on one kidney only and they underwent unilateral nephrectomy. Normal control blood samples were collected from healthy male individuals (n = 11), which were within the same age group with the patients. The blood samples were then transferred into clean, sterile centrifuge tubes and allowed to clot. Each clotted sample was centrifuged at 3000 g for 10 min to obtain the serum. Enzyme assay was carried out within 24 h of collection.

Enzyme Assays

The serum activities of the three lysosomal enzymes were assayed fluorometrically by estimating the amount of 4-methylumbelliferone released from the appropriate substrate. The β-glucuronidase activity was determined in a reaction medium that contained 10 mM 4-methylumbelliferyl-β-D-glucuronide in 1 M sodium acetate buffer (pH 4.8). The β-galactosidase activity was determined using an incubation medium which contained 3.33 mM 4-methylumbelliferyl-β-D-galactoside in citrate-phosphate buffer with 0.2 M sodium chloride (pH 4.4). The acid phosphatase activity was determined using an incubation medium which contained 10 mM 4-methylumbelliferyl-phosphate in 1 M sodium acetate buffer (pH 4.5). Serum (10 μL) was added to 90 μL of incubation mixture for each enzyme assay. All assays were performed at 37°C; The incubation time being 30 min for β-glucuronidase and β-galactosidase and 15 min for acid phosphatase. The reactions were terminated by adding 2.9 mL of 0.3 M glycine/ammonia buffer (pH 10.0), to make a final volume of 3.0 mL.

The amount of 4-methylumbelliferone liberated was measured fluorometrically as described elsewhere (Adoga and Glew, 1995; Gatsing *et al*., 2000, 2002, 2003; Gatsing and Adoga, 2004) using a Turner fluorometer, model III (Sequoia-Turner Corp., Mountain View, CA), with excitation at 360 nm and emission at 520-580 nm. The assays are highly reproducible and have a standard error of

less than 4% (VanderJagt *et al*., 1992; Adoga and Glew, 1995; Yazzie *et al*., 1995; Gatsing *et al*., 2000; Gatsing *et al*., 2002; Gatsing *et al*., 2003; Gatsing and Adoga, 2004; Gatsing *et al*., 2006). Serum enzyme activities were expressed as nmole/h/mL serum. Results for both the patients and the controls are expressed as the mean±1 standard deviation (SD).

Statistical Analysis

Statistical analyses were performed with the aid of the Number Cruncher Statistical System software program (NCSS, version 5, Kaysville, UT). Group comparisons were done using the Mann-Whitney two-sample test and One-way analysis of variance (ANOVA). A p-value of <0.05 was considered statistically significant.

Ethics

This work was conducted in accordance with the following ethical declarations:

- World Medical Association's Declaration of Helsinki (WMA, 1996).
- APA Ethical Principles in the Conduct of Research with Human Participants (APA, 1982).
- World Medical Association's Declaration of Lisbon on the Rights of the Patient (WMA, 1995).
- CIOMS/WHO International Guidelines for the Conduct of Research Involving Human Subjects (CIOMS/WHO, 1993).

Moreover, samples were obtained after the research protocols had been approved by the Department of Surgery at JUTH. Besides, informed consent was obtained from one of the relations of each patient after explanation of the purpose of the study.

RESULTS

Patients with Wilms' tumour showed a significant ($p<0.05$) increase in β-glucuronidase activity before surgical operation, as compared to control values. This was followed by significant ($p<0.05$) decreases in β-glucuronidase activities 24, 72 h and 7 days after surgical intervention, as compared to pre-operation values. The activity of β-glucuronidase observed 7 days after operation was still significantly ($p<0.05$) higher than the control value (Table 1).

β-Galactosidase activity showed a slight increase before operation, but which was not statistically significant ($p>0.05$), as compared to control value. This was followed by a slight decrease 24 h after operation, which still was not statistically significant ($p>0.05$), as compared to pre-operation value.

Acid phosphatase activity showed a decrease before operation, but which was not statistically significant ($p>0.05$), as compared to control value. The activity of this enzyme was found to be increased after surgical intervention, but still this increase was not statistically significant ($p>0.05$), as compared to pre-operation value (Table 1).

Table 1: Serum activities of some lysosomal enzymes in Wilms' tumour patients before and after surgical operation

	Enzyme Activity: nmole/h/mL serum		
Time	β-Glucuronidase	β-Galactosidase	Acid phosphatase
Controls	257.8±72.2	235.5±69.3	462.4±70.8
Pre-operation	979.7±106.9[a]	314.3±43.5	369.4±58.7
24 h post-operation	803.5±52.3[a, b]	224.9±57.8	395.8±61.0
72 h post-operation	492.1±63.2[a, b]	406.6±125.4	398.0±59.1
7 days post-operation	401.3±46.4[a, b]	395.0±95.1	390.2±30.0

Tabulated values are Mean±SD of eleven independent determinations, Key: a: $p<0.05$ vs control; b: $p<0.05$ vs pre-operation values

DISCUSSION

Not much work has been done on the effect of the disease and surgery on the serum levels of lysosomal enzymes in nephroblastoma. However, it is known that kidney damage leads to the urinary excretion of abnormally high activities of β-hexosaminidase and β-galactosidase (Price *et al*., 1970; Wellwood *et al*., 1976), as has been shown for rats injected with nephrotoxic agents (Dance *et al*., 1970). Urinary β-glucuronidase has been found to be a useful marker of renal injury and disease (Kunin *et al*., 1978; Plummer *et al*., 1986) because of its high molecular weight and presence in high concentrations in the kidney (Yazzie *et al*., 1995).

Proliferation of cells, an increase in the rate of cell turnover, cell damage or an increase in the rate of enzyme induction and synthesis and their reduced clearance result in increased serum/plasma enzyme concentrations (Balami *et al*., 1998).

The data obtained in the present study revealed that in nephroblastoma patients, serum activity of β-galactosidase showed a slight increase before operation and a slight decrease after operation, all of which were not statistically significant as compared to control and pre-operation values, respectively. In contrast, acid phosphatase activity showed a decrease before operation and an increase after operation, all of which still were not statistically significant as compared to control and pre-operation values, respectively. This result suggests that the disease and surgery may have a selective effect on lysosomal enzymes.

The most interesting result here was the finding of a significant increase in β-glucuronidase activity in the serum of patients before operation as compared to control values (257.8±72.2 for control and 979.7±106.9 for patients); The pre-operation values being about 4-fold higher than the control values. The medical report did not show any metastases in these patients. An increased activity of lysosomal enzymes in the blood is indicative of pathologic changes on tissues (Raulo *et al*., 1996). The increased release of β-glucuronidase into the bloodstream of nephroblastoma patients may therefore be due to tissue damage or necrosis created by the disease. This may also be due to the effect of the underlying pathological condition on the state of the lysosomal membrane. Besides, β-glucuronidase serum activity showed significant decreases 24, 72 h and 7 days after operation as compared to pre-operation values. Also, the medical report indicated some improvement in the patients' conditions after surgical intervention. A decrease in the blood level of this enzyme after operation may therefore indicate recovery from the disease. Hence, the variations in β-glucuronidase activity in the serum may prove valuable in monitoring progress in nephroblastoma.

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